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Brief Report

Field survey of reproductive modes and sodium channel mutations associated with pyrethroid resistance in *Thrips tabaci*

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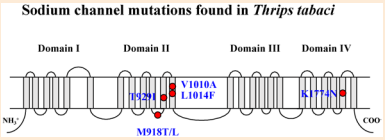
Using PCR-Restriction Fragment Length Polymorphism (RFLP) with mitochondrial cytochrome *c* oxidase subunit I sequences, we examined the reproductive modes of female adults of *Thrips tabaci* collected at 54 sites across Japan. Results showed the presence of heteroplasmic insects harboring mitochondria associated with arrhenotoky and thelytoky. Using the insects, we also applied PCR-RFLP to examine the genotypes for the amino acid mutation (T929I) site involved in pyrethroid resistance. Findings showed the presence of thelytokous heterozygotes under the circumstance that most arrhenotokous insects are resistant homozygotes, and many thelytokous insects are susceptible homozygotes. These results suggest that, in the field, genetic exchange occurs between insects through of both reproductive modes. A survey of the genotypes for the other amino acid mutations using nucleotide sequencing showed a decline of insects with an M918T and L1014F pair and an increase of insects with M918L. These results suggest the evolutional progression of amino acid mutations associated with pyrethroid resistance in *T. tabaci*.



Keywords: arrhenotoky, onion thrips, insecticide resistance, target insensitivity, thelytoky.

Introduction

Thrips tabaci Lindeman (Thysanoptera: Thripidae) has been recognized as a destructive agricultural pest species worldwide.[1)] The thrips directly suck cell fluids from leaves, stems, flowers, and the surfaces of fruits of widely various crops, thereby causing silvery scarring and leaf chlorosis, which reduce their commercial value considerably. Moreover, they transmit *Tomato spotted wilt virus* (TSWV)[2)] and *Iris yellow spot virus* (IYSV)[3)] in a persistent manner, widening the scale and scope of economic damage.

Three reproductive modes have been reported in *T. tabaci*: arrhenotoky, thelytoky, and duterotoky.[4,5)] Arrhenotoky is a form of parthenogenesis by which unfertilized eggs develop into haploid males.[4)] With thelytoky, only females develop from unfertilized eggs.[4)] Duterotoky, by which females and males appear from unfertilized eggs, has been reported in the United States,[5)] but not in Japan.[6,7)]

Thrips tabaci has developed resistance to various insecticides including pyrethroids. Pyrethroid resistance of *T. tabaci* is conferred mainly by target insensitivity of the sodium channel caused by amino acid mutations. Six amino acid mutations have been correlated with the resistance: M918T, M918L, T929I, V1010A, L1014F, and K1774N.[6–11)] Thelytokous insects with M918T and L1014F exhibit a high level of resistance to a pyrethroid.[8)] Arrhenotokous insects with T929I show a moderate level of pyrethroid resistance.[8)] Jouraku *et al.*[10)] reported that T929I is linked with K1774N and that insects with both mutations show a high degree of pyrethroid resistance. Recently, the presence of thelytokous insects with M918L was reported in Japan.[10)] However, the distribution of insects with amino acid mutations and their reproductive modes described above have not been investigated extensively in the field.

Earlier, we showed that all arrhenotokous insects were homozygous for the T929I site, whereas only some thelytokous insects were homozygous for the mutation site.[7)] Arrhenotokous and thelytokous insects sometimes inhabit the same site[7)] and mate irrespective of the reproductive mode of their counterpart.[12)] Therefore, genetic exchange might occur through hybridization

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between insects of both reproductive modes in the field. In fact, genetic exchange between both reproductive modes has been shown to occur with low frequency.[13,14] Nevertheless, insects heterozygous for the T929I site were not observed in our earlier study.[7]

For this study, we examine genotypes for the sodium channel mutation sites and reproductive modes using larger numbers of female adults of *T. tabaci* collected from various regions of Japan. Based on these data, the possibility of genetic exchange between arrhenotoky and thelytoky and the evolutional progression of the sodium channel mutations are discussed.

Materials and methods

1. Insect collection and genomic DNA extraction

Thrips tabaci individuals were collected in the field from 2021 through 2023 (Table 1). Collected insects were stored in 99.5% ethanol or at −80°C until DNA extraction.

Genomic DNA was extracted from a single female adult using MightyPrep reagent for DNA (Takara Bio Inc., Kusatsu, Japan). Briefly, a single insect introduced into 10 μL of the reagent was incubated at 95°C for 10 min and then at room temperature for 2 min. After centrifugation at 15,000×*g* for 2 min, the supernatant was recovered as a DNA sample. For subsequent PCR amplification, 0.5 μL of the supernatant was used.

2. Determination of reproductive mode

The reproductive mode was examined based on the nucleotide sequences of the cytochrome *c* oxidase subunit I (COI) gene. A part of the COI sequence was amplified by PCR using the primers UEA3 (5′-tatagcattcccacgaataaataa-3′)[15] and Tt448 (5′-atgagaaattagtccaaatcctgg-3′).[16] The PCR conditions were 1 cycle of 3 min at 94°C, 40 cycles of 15 sec at 94°C, 30 sec at 50°C, and 1 min at 72°C, with final extension of 72°C for 7 min. EmeraldAmp MAX PCR Master Mix (Takara Bio Inc.) was used for PCR amplification. Digestion of the amplified DNA fragments with *Eco*O109I produces restriction fragments with lengths of 350 bp and 140 bp for arrhenotoky and restriction fragments with lengths of 260 bp, 140 bp, and 90 bp for thelytoky. The digested DNA fragments were confirmed by 1.5% or 2% agarose gel electrophoresis. We applied this method for reproductive mode determination of our cultured arrhenotokous (KOC16, KOC2442, TOK401, KAG4-5, and KTF-SPRR) and thelytokous (KAG1, KOC50, KOC2, and KOC2-2) strains[11] and found that the method correctly judged their reproductive modes, except for arrhenotokous KOC16 strain with thelytokous-associated COI sequence.[17] An arrhenotokous strain (KYT-M1) with thelytokous-associated COI sequence was also reported in another study.[11] Nevertheless, the frequency of such insects is very low, and this method is useful as an initial screen for the reproductive modes of *T. tabaci*.

DNA fragments amplified from two insects collected in Hokkaido prefecture showing restriction patterns of both reproductive modes were cloned into pMD20 vector (Takara Bio Inc.) according to the manufacturer's recommendations. Then they were sequenced.

3. Genotyping of sodium channel mutation sites

The genomic DNA fragments corresponding to domains IIS4-IIS6 of the sodium channel gene were amplified using PCR with the primers Tt-Na-5′-3 (5′-tgagtccgaagttctatttt-3′) and Tt-Na-3′-5 (5′-ggtccgagatctgattcgtc-3′).[6] The PCR conditions were 1 cycle of 3 min at 94°C, 40 cycles of 15 sec at 94°C, 30 sec at 60°C, and 1 min at 72°C, with final extension of 72°C for 7 min. EmeraldAmp MAX PCR Master Mix was used for PCR amplification. Digestion of the amplified DNA fragments with *Mbo*I produces restriction fragments with lengths of 519 bp and 216 bp for susceptible homozygotes and restriction fragments with lengths of 287 bp, 232 bp, and 216 bp for resistant homozygotes. All the described restriction fragments (519 bp, 287 bp, 232 bp, and 216 bp) are produced in heterozygotes. The digested DNA fragments were confirmed using 1.5% or 2% agarose gel electrophoresis.

A certain number of heterozygotes and susceptible homozygotes for the T929I site were subjected to genotyping for the amino acid mutation (M918T, M918L, V1010A, and L1014F) sites. For this genotyping, the amplified sodium channel DNA fragments were sequenced directly using the Tt-Na-direct-seq4 (5′-gcgaacgtttgctttgatcc-3′) primer, as described by Aizawa *et al.*[6]

4. Nucleotide sequencing

Nucleotide sequencing was conducted using a dye terminator cycle sequencing kit (ver. 3.1; Applied Biosystems, Waltham, MA, USA) and a DNA sequencer (3500 Genetic Analyzer; Applied Biosystems). Nucleotide and deduced amino acid sequences were analyzed using software (Genetyx ver. 13; Genetyx Corp., Tokyo, Japan).

Results

1. Determination of reproductive mode

In all, 2,277 female adults were collected at 54 sites from northern to southern Japan and were examined for their reproductive modes. Results showed that 1,068 and 1,178 insects, respectively, were classifiable as arrhenotoky and thelytoky (Table 1). Our PCR-RFLP method failed to indicate the reproductive mode of the remaining 31 insects because restriction patterns of both reproductive modes were observed (The restriction patterns of three out of 31 insects shown in lanes 1, 2, and 7 in Fig. 1). Those insects were detected at four study sites: Tochigi 2, Hokkaido 1, Akita 2, and Wakayama 3. Of the 298 insects collected at the study sites with no pesticide application (Kumamoto 1, Kumamoto 2, Shimane 1, Shimane 2, Miyazaki, Okayama, and Hyogo 1), 11 and 287 were, respectively, classifiable as arrhenotoky and thelytoky.

To assess the possibility of heteroplasmy in 31 insects showing restriction patterns of both reproductive modes, the COI fragments amplified from two insects collected at Hokkaido 1 were cloned and sequenced. For one insect, 11 clones were examined. Among the 11 clones, 10 showed arrhenotokous-associated se-

Table 1. Genotypes for the T929I site in female adults of *Thrips tabaci* examined in this study.

Study site	*n*	Reproductive mode									Host plant	Location (Latitude and longitude of municipality)	Month, Year	Note
		Arrhenotoky			Thelytoky			Unknown						
		RR	*RS*	*SS*	*RR*	*RS*	*SS*	*RR*	*RS*	*SS*				
Hokkaido 1	271	176	7	1	3	6	56	2	11	9	*A. cepa*	Naganuma town, Hokkaido pref (N43°00′ E141°41′)	July, 2021	
Hokkaido 2	76	0	0	0	0	0	76	0	0	0	*A. cepa*	Kunneppu town, Hokkaido pref (N43°43′ E143°45′)	July, 2023	
Akita 1	31	31	0	0	0	0	0	0	0	0	*A. fistulosum*	Noshiro city, Akita pref (N40°12′ E140°01′)	September, 2021	
Akita 2	76	66	1	0	1	0	1	2	4	1	*A. fistulosum*	Happo town, Akita pref (N40°19′ E140°02′)	September, 2021	
Iwate 1	39	29	0	0	0	0	10	0	0	0	*A. fistulosum*	Morioka city, Iwate pref (N39°70′ E141°15′)	September, 2022	
Iwate 2	7	0	0	0	0	0	7	0	0	0	*A. fistulosum*	Ohta, Hanamaki city, Iwate pref (N39°38′ E141°11′)	September, 2022	
Iwate 3	28	0	0	0	0	0	28	0	0	0	*A. fistulosum*	Shiba town, Iwate pref (N39°33′ E141°09′)	September, 2022	
Iwate 4	28	0	0	0	0	0	28	0	0	0	*A. fistulosum*	Nishiwaga town, Iwate pref (N39°19′ E140°46′)	September, 2022	
Iwate 5	35	0	0	0	0	0	35	0	0	0	*A. fistulosum*	Ohata, Hanamaki city, Iwate pref	September, 2022	
Yamagata 1	31	29	0	0	2	0	0	0	0	0	*A. fistulosum*	Sagae city, Yamagata pref (N38°22′ E140°16′)	July, 2022	
Yamagata 2	28	26	0	0	2	0	0	0	0	0	*A. fistulosum*	Shonai town, Yamagata pref (N38°50′ E139°54′)	August, 2022	
Miyagi 1	64	0	0	0	0	1	63	0	0	0	*A. cepa*	Shibata town, Miyagi pref (N38°03′ E140°45′)	July, 2022	
Miyagi 2	116	31	0	0	0	0	85	0	0	0	*A. fistulosum, A. cepa*	Natori city, Miyagi pref (N38°10′ E140°53′)	July, 2022	
Tochigi 1	67	59	0	0	0	1	7	0	0	0	*Allium fistulosum*	Kamikomoriya, Utsunomiya city, Tochigi pref (N36°33′ E139°52′)	June, 2021	
Tochigi 2	67	57	0	0	0	0	9	0	1	0	*A. fistulosum*	Mooka city, Tochigi pref (N36°44′ E140°01′)	June, 2021	
Tochigi 3	44	2	0	0	0	0	42	0	0	0	*A. fistulosum*	Minemachi, Utsunomiya city, Tochigi pref	May, 2022	
Chiba 1	45	43	0	0	0	0	2	0	0	0	*A. fistulosum*	Arai, Yokoshibahikari town, Chiba pref (N35°39′ E140°30′)	June, 2021	
Chiba 2	95	95	0	0	0	0	0	0	0	0	*A. fistulosum*	Haratkata, Yokoshibahikari town, Chiba pref	June, 2021	
Chiba 3	42	32	1	0	0	0	9	0	0	0	*A. fistulosum*	Oamishirosato city, Chiba pref (N35°31′ E140°19′)	June, 2021	
Chiba 4	35	35	0	0	0	0	0	0	0	0	*A. fistulosum*	Kokubun, Ichikawa city, Chiba pref (N35°71′ E139°92′)	August, 2021	
Chiba 5	33	33	0	0	0	0	0	0	0	0	*A. fistulosum*	Kitakokubun, Ichikawa city, Chiba pref	August, 2021	
Chiba 6	49	45	0	0	0	1	3	0	0	0	*A. cepa*	Arai, Yokoshibahikari town, Chiba Pref	June, 2022	
Chiba 7	16	11	0	0	0	0	5	0	0	0	*A. cepa*	Haratkata, Yokoshibahikari town, Chiba Pref	June, 2022	
Yamanashi 1	38	37	1	0	0	0	0	0	0	0	*A. fistulosum*	Iwamori, Kai city, Yamanashi pref (N35°39′ E138°30′)	July, 2022	
Yamanashi 2	54	11	28	6	5	1	3	0	0	0	*A. fistulosum*	Shimoimai, Kai city, Yamanashi pref	July, 2022	
Nagano 1	45	43	1	0	1	0	0	0	0	0	*A. fistulosum*	Yamagata village, Nagano pref (N36°10′ E137°52′)	October, 2022	
Nagano 2	48	47	0	0	0	0	1	0	0	0	*A. fistulosum*	Matsumoto city, Nagano pref (N36°14′ E137°58′)	October, 2022	
Kyoto 1	18	0	0	0	2	1	15	0	0	0	*Daucus carota* subsp. Sativus	Kyotamba town, Kyoto Pref (N35°10′ E135°25′)	December, 2020 and January, 2021	
Kyoto 2	14	11	1	0	2	0	0	0	0	0	*A. fistulosum*	Takeda, Fushimi-ku, Kyoto city, Kyoto pref (N35°01′ E135°76′)	June, 2022	
Kyoto 3	9	6	0	0	2	0	1	0	0	0	*A. fistulosum*	Mukaijima, Fushimi-ku, Kyoto city, Kyoto pref	June, 2022	
Osaka	15	0	0	0	0	0	15	0	0	0	*A. officinalis*	Habikino city, Osaka pref (N34°55′ E135°57′)	July, 2023	
Wakayama 1	8	5	0	0	0	0	3	0	0	0	*Allium cepa*	Gobo city, Wakayama pref (N33°89′ E135°15′)	April, 2022	
Wakayama 2	49	25	2	0	0	1	21	0	0	0	*A. cepa*	Nishikawahara, Kinokawa city, Wakayama pref (N34°26′ E135°36′)	April, 2022	

Table 1. Continued

Study site	*n*	Reproductive mode									Host plant	Location (Latitude and longitude of municipality)	Month, Year	Note
		Arrhenotoky			Thelytoky			Unknown						
		RR	*RS*	*SS*	*RR*	*RS*	*SS*	*RR*	*RS*	*SS*				
Wakayama 3	53	12	0	1	5	2	32	1	0	0	*A. cepa*	Kirihata, Kinokawa city, Wakayama pref	April, 2022	
Wakayama 4	51	10	0	0	0	0	41	0	0	0	*A. cepa*	Kokawa, Kinokawa city, Wakayama pref	April, 2022	
Hyogo 1	53	0	0	0	1	0	52	0	0	0	*A. fistulosum*	Kasai city, Hyogo pref (N34°55′ E134°50′)	May, 2022	No pesticide use
Hyogo 2	64	0	0	0	0	0	64	0	0	0	*A. cepa*	Awaji city, Hyogo pref (N34°26′ E134°54′)	May, 2022	
Okayama	51	0	0	0	0	0	51	0	0	0	*A. cepa, B. oleracea* var. capitata	Okayama city, Okayama pref (N34°65′ E133°91′)	May, 2022	No pesticide use
Hiroshima 1	7	0	0	0	7	0	0	0	0	0	*Asparagus officinalis*	Funocho, Miyoshi city, Hiroshima pref (N34°48′ E132°51′)	June, 2022	
Hiroshima 2	20	0	0	0	0	0	20	0	0	0	*A. officinalis*	Miwacho, Miyoshi city, Hiroshima pref	June, 2022	
Hiroshima 3	37	0	0	0	7	0	30	0	0	0	*A. officinalis*	Kawashiri, Sera town, Hiroshima pref (N34°35′ E133°03′)	June, 2022	
Hiroshima 4	13	0	0	0	0	0	13	0	0	0	*A. officinalis*	Uzuto, Sera town, Hiroshima pref	June, 2022	
Hiroshima 5	14	0	0	0	4	0	10	0	0	0	*Solanum melongena*	Higashihiroshima city, Hiroshima pref (N34°25′ E132°44′)	July, 2022	
Hiroshima 6	7	0	0	0	0	1	6	0	0	0	*Eustoma grandiflorum*	Higashihiroshima city, Hiroshima pref	July, 2022	
Hiroshima 7	14	0	0	0	1	0	13	0	0	0	*A. officinalis*	Miyoshi city, Hiroshima pref	July, 2022	
Shimane 1	51	5	0	0	0	0	46	0	0	0	*A. cepa*	Hikawa town, Izumo city, Shimane pref (N35°22′ E132°45′)	May, 2022	No pesticide use
Shimane 2	51	1	0	0	0	0	50	0	0	0	*A. cepa*	Izumo city, Shimane pref	May, 2022	No pesticide use
Nagasaki 1	21	0	0	0	0	0	21	0	0	0	*A. officinalis*	Isahaya city, Nagasaki pref (N32°84′ E130°05′)	July, 2022	
Nagasaki 2	10	0	0	0	3	0	7	0	0	0	*A. officinalis*	Shimabara city, Nagasaki pref (N32°78′ E130°37′)	October, 2022	
Nagasaki 3	38	0	0	0	28	1	9	0	0	0	*A. officinalis*	Higashisonogi town, Nagasaki pref (N33°02′ E129°55′)	October, 2022	
Nagasaki 4	9	0	0	0	0	0	9	0	0	0	*A. officinalis*	Nagasaki city, Nagasaki pref (N32°75′ E129°87′)	August, 2022	
Kumamoto 1	32	0	0	0	0	0	32	0	0	0	*A. fistulosum*	Yatsushiro city, Kumamoto pref (N32°50′ E130°60′)	May, 2022	No pesticide use
Kumamoto 2	15	2	0	0	0	0	13	0	0	0	*A. fistulosum, Allium sativum*	Koshi city, Kumamoto pref (N32°53′ E130°77′)	May, 2022	No pesticide use
Miyazaki	45	3	0	0	0	1	41	0	0	0	*Brassica oleracea* var. capitata	Miyazaki city, Miyazaki pref (N31°90′ E131°42′)	May, 2022	No pesticide use

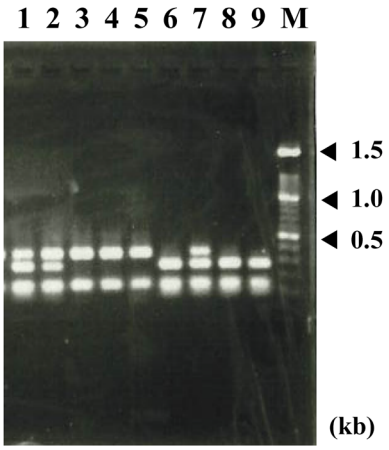

Fig. 1. Restriction patterns of the COI gene fragments amplified from *Thrips tabaci*. After the COI gene fragments amplified by PCR were digested with *Eco*O109I, they were size fractionated on 2% agarose gel. Restriction patterns for arrhenotoky and thelytoky were observed, respectively, in lanes 3, 4, and 5 and in lanes 6, 8, and 9. In lanes 1, 2, and 7, restriction patterns for both reproductive modes were observed.

quences, whereas the remaining clone was thelytokous-associated (data not shown). For another insect, six examined clones were also classified into two sequences: arrhenotokous-associated (three clones) and thelytokous-associated (three clones). These results suggest the presence of heteroplasmic insects having mitochondria associated with both reproductive modes in *T. tabaci*.

2. Genotyping of sodium channel mutation sites

The results of genotyping for the T929I site are presented in Table 1. Insects with T929I were detected throughout Japan. Most of the arrhenotokous female adults were resistant homozygotes for the T929I site. In arrhenotoky, susceptible homozygotes (eight insects) and heterozygotes (42 insects) were, respectively, detected at three study sites (Hokkaido 1, Wakayama 3, and Yamanashi 2) and at eight study sites (Chiba 3, Hokkaido 1, Akita 2, Wakayama 2, Kyoto 2, Yamanashi 1, Yamanashi 2, and Nagano 1). On the other hand, many of the thelytokous female adults were susceptible homozygotes for the T929I site. Regarding thelytoky, a total of 17 heterozygous insects were detected

Table 2. Genotypes for the M918L, M918T, and L1014F sites in heterozygotes and susceptible homozygotes for the T929I site in *Thrips tabaci*.

Study site	Thelytoky										Arrhenotoky										Unknown									
	n	M918L			M918T			L1014F			*n*	M918L			M918T			L1014F			*n*	M918L			M918T			L1014F		
		RR	*RS*	*SS*	*RR*	*RS*	*SS*	*RR*	*RS*	*SS*		*RR*	*RS*	*SS*	*RR*	*RS*	*SS*	*RR*	*RS*	*SS*		*RR*	*RS*	*SS*	*RR*	*RS*	*SS*	*RR*	*RS*	*SS*
Hokkaido 1	59	0	55	4	0	0	59	0	0	59	8	0	3	5	0	0	8	0	0	8	20	0	10	10	0	0	20	0	0	20
Hokkaido 2	76	0	7	69	0	2	74	0	2	74	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Akita 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Akita 2	1	0	0	1	0	0	1	0	0	1	1	0	0	1	0	0	1	0	0	1	5	0	0	5	0	0	5	0	0	5
Iwate 1	10	0	0	10	0	0	10	0	0	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Iwate 2	7	0	0	7	0	0	7	0	0	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Iwate 3	12	0	0	12	0	0	12	0	0	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Iwate 4	12	0	0	12	0	0	12	0	0	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Iwate 5	11	0	0	11	0	0	11	0	0	11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Yamagata 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Yamagata 2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Miyagi 1	17	0	0	17	0	0	17	0	0	17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Miyagi 2	31	0	0	31	0	0	31	0	0	31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tochigi 1	8	0	0	8	0	0	8	0	0	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tochigi 2	9	0	0	9	0	0	9	0	0	9	—	—	—	—	—	—	—	—	—	—	1	0	0	1	0	0	1	0	0	1
Tochigi 3	13	0	0	13	0	0	13	0	0	13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chiba 1	2	0	0	2	0	0	2	0	0	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chiba 2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chiba 3	8	0	0	8	0	0	8	0	0	8	1	0	0	1	0	0	1	0	0	1	—	—	—	—	—	—	—	—	—	—
Chiba 4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chiba 5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chiba 6	4	0	0	4	0	0	4	0	0	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chiba 7	5	0	0	5	0	0	5	0	0	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Yamanashi 1	—	—	—	—	—	—	—	—	—	—	1	0	0	1	0	0	1	0	0	1	—	—	—	—	—	—	—	—	—	—
Yamanashi 2	—	—	—	—	—	—	—	—	—	—	29	0	0	29	0	0	29	0	0	29	—	—	—	—	—	—	—	—	—	—
Nagano 1	—	—	—	—	—	—	—	—	—	—	1	0	0	1	0	0	1	0	0	1	—	—	—	—	—	—	—	—	—	—
Nagano 2	1	0	0	1	0	0	1	0	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kyoto 1	16	0	0	0	0	0	16	0	0	16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kyoto 2	—	—	—	—	—	—	—	—	—	—	1	0	0	1	0	0	1	0	0	1	—	—	—	—	—	—	—	—	—	—
Kyoto 3	1	0	0	1	0	0	1	0	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Osaka	14	0	0	14	0	0	14	0	0	14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Wakayama 1	3	0	1	2	0	0	3	0	0	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Wakayama 2	22	0	0	22	0	0	22	0	0	22	2	0	0	2	0	0	2	0	0	2	—	—	—	—	—	—	—	—	—	—
Wakayama 3	34	0	0	34	0	0	34	0	0	34	1	0	0	1	0	0	1	0	0	1	—	—	—	—	—	—	—	—	—	—
Wakayama 4	40	0	0	40	0	0	40	0	0	40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hyogo 1	14	0	0	14	0	0	14	0	0	14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hyogo 2	7	0	0	7	0	0	7	0	0	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Okayama	16	0	0	16	0	0	16	0	0	16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hiroshima 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hiroshima 2	6	0	0	6	0	0	6	0	0	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hiroshima 3	16	0	0	16	0	0	16	0	0	16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hiroshima 4	4	0	0	4	0	0	4	0	0	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hiroshima 5	10	0	0	10	0	0	10	0	0	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hiroshima 6	7	0	0	7	0	0	7	0	0	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hiroshima 7	13	0	0	13	0	0	13	0	0	13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Shimane 1	46	0	0	46	0	0	46	0	0	46	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Shimane 2	15	0	0	15	0	0	15	0	0	15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nagasaki 1	15	0	0	15	0	0	15	0	0	15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nagasaki 2	7	0	0	7	0	0	7	0	0	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nagasaki 3	10	0	0	10	0	0	10	0	0	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nagasaki 4	8	0	0	8	0	0	8	0	0	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kumamoto 1	14	0	0	14	0	0	14	0	0	14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kumamoto 2	13	0	0	13	0	0	13	0	0	13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Miyazaki	16	0	0	16	0	0	16	0	0	16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

at 11 study sites: Tochigi 1, Hokkaido 1, Kyoto 1, Wakayama 2, Wakayama 3, Miyazaki, Chiba 6, Miyagi 1, Yamanashi 2, Hiroshima 6, and Nagasaki 3. Regarding the heterozygous insects, the results of genotyping using PCR-RFLP were confirmed by nucleotide sequencing, except for one insect collected at Yamanashi 2 (data not shown).

The results of genotyping for mutation sites other than the T929I site are presented in Table 2. Thelytokous insects with a pair of M918T and L1014F were detected at a very low frequency (two insects) at only one study site (Hokkaido 2). They encoded the mutations heterozygously as reported by Toda and Morishita[8] (data not shown). On the other hand, insects heterozygously encoding M918L were found at three study sites (Hokkaido 1, Wakayama 1, and Hokkaido 2). Among them, three arrhenotokous insects collected at Hokkaido 1 were heterozygous for both M918L and T929I sites. If the reproductive mode predicted by the PCR-RFLP method was correct, then this report is the first of insects with M918L in arrhenotoky. The linkage examination between T929I and M918L and other amino acid mutations remained in the future. No insect with V1010A was detected in this study (data not shown).

Discussion

In recent years, decreased susceptibility to multiple insecticides has been reported for *T. tabaci*.[18–20] However, decreased susceptibility to organophosphates, pyrethroids, neonicotinoids, and diamides has been reported only in the arrhenotokous populations.[19–22] Arrhenotokous and thelytokous strains with T929I exhibited a similar level of resistance to a pyrethroid, but biotic performances such as longevity and fecundity were markedly inferior in the latter.[6,7] Little or no information related to the fitness costs of resistance to organophosphates, neonicotinoids, and diamides in different reproductive modes of *T. tabaci* has been available. However, considering the ratio of arrhenotokous and thelytokous insects at study sites with and without pesticide applications, the fitness costs to the insecticides in arrhenotokous insects might be lower than those in thelytokous insects. Future studies must examine details of the relation between insecticide resistance and fitness costs.

Heteroplasmy, the existence of mitochondrial gene variants within a single cell or individual, might occur through some mechanisms, including paternal leakage.[23,24] From the present study, we found heteroplasmic insects exhibiting the presence of mitochondria associated with both reproductive modes. Gawande *et al.*[25] reported that heteroplasmic insects might have resulted from the introgression of mitochondria of arrhenotokous males into thelytokous females through paternal leakage during mating. If their inference is correct, then the reproductive mode of heteroplasmic insects must be thelytoky. The reproductive modes of the heteroplasmic insects identified in this study were unclear. The establishment of heteroplasmic strains and examination of their reproductive modes remain as objectives for future investigations.

Most of the arrhenotokous insects were resistant homozygotes for the T929I site, whereas many thelytokous insects were susceptible homozygotes (Table 1). They coexisted at 26 study sites out of 54. The present study detected, for the first time reported, thelytokous insects heterozygously encoding T929I at 11 study sites across 10 prefectures. Reportedly, arrhenotokous males mated not only with arrhenotokous females but also with thelytokous females.[12] Gene transfer from arrhenotokous males to thelytokous females has been shown to occur with low frequency.[13,14] Consequently, the thelytokous insects heterozygously encoding T929I might have resulted through mating between arrhenotokous insects with T929I and thelytokous insects without the mutation. In an earlier study, we demonstrated that *T. tabaci* is divisible into three groups based on the COI sequence: diploid thelytoky, triploid thelytoky, and diploid arrhenotoky.[11] In addition, our principal component analysis using whole genome resequencing data demonstrated that diploid and triploid thelytokous groups are further classifiable into two based on the sodium channel mutations harbored by respective group members (strains).[11] Based on the results of subsequent admixture analysis, we inferred that a thelytokous group without T929I might be a recipient of gene flow from arrhenotokous males.[11] Mating experiments must be conducted to clarify the genetic background of arrhenotokous and thelytokous insects that enable genetic exchange between different reproductive modes.

It is of particular interest that arrhenotokous insects without T929I were detected in this study. In Japan, thelytokous insects were dominant.[26,27] In the late 1990s to early 2000s, *T. tabaci* began to damage a wider range of agricultural crops[28–32] and exhibited decreased insecticide susceptibility.[28,32,33] Arrhenotokous insects began to be observed frequently during the period.[8] All arrhenotokous strains examined had T929I.[7,34] Based on the observations, it has been speculated that arrhenotokous insects with T929I invaded from overseas.[35,36] However, the discovery of arrhenotokous insects without T929I in this study suggests that arrhenotokous insects susceptible to pyrethroids had invaded together with the resistant insects of the same reproductive mode but they have been eliminated through selection with pyrethroids.

Thelytokous insects with a pair of M918T and L1014F were observed with a certain frequency in 2001–2010.[8,34] However, those insects have not been observed in the past few years.[6,10] From this study, we confirmed the presence of insects with a pair of M918T and L1014F, but their frequency was very low. We also confirmed the increase of insects with M918L. These results suggest the evolutional progression of amino acid mutations associated with pyrethroid resistance. Surveys to elucidate the contribution of these mutations to pyrethroid resistance and fitness costs and their future distribution are expected to be valuable for more effective management of *T. tabaci*.

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Declaration of interest

none

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