[SHORT COMMUNICATION]

Comparison of Genetic Diversity among Three Phytoseiid Mite Species in Japan by Mitochondrial DNA Sequence Analysis

Norihide HINOMOTO^{1*}, Toshiyuki SHINTAKU² and Hiroshi AMANO^{2†}

¹*Natural Enemies Laboratory, Insect Interaction Research Unit, Division of Insect Sciences, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8634, Japan*

²*Laboratory of Applied Entomology and Zoology, Graduate School of Horticulture, Chiba University, Matsudo, Chiba 271-8510, Japan*

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INTRODUCTION

The nontarget effects of biocontrol agents introduced to biological control programs have been discussed widely (e.g., van Lenteren et al., 2003; Louda et al., 2003; Simberloff and Stiling, 1996). These discussions have often focused on whether the agents used are native or exotic. Biological control of agricultural pests is used to avoid the environmental pollution caused by intensive chemical sprays. However, it still carries a risk of ecological disturbance: if the introduced predator or parasitoid directly or indirectly competes with native species, then there is a great risk of species replacement (Howarth, 1991; Samways, 1997; van Lenteren et al., 2003).

Neoseiulus californicus (McGregor) (Acari: Phytoseiidae) is a cosmopolitan predatory mite in the subtropical and temperate regions of Europe and North and South America (McMurtry and Croft, 1997), as well as in Japan. In 1963 it was recorded from Tokyo for the first time in Japan (Ehara, 1964). Before the 1980s few specimens were collected, but by the 1990s the numbers of *N. californicus* collected from agricultural fields and surrounding areas had increased markedly (Amano et al., 2004), and this species had become a dominant and well-established predator of spider mites in the orchards and groves of central Japan (Katayama et al., 2006; Kishimoto, 2002).

A commercial strain of *N. californicus* was first imported into Japan from the Netherlands in

日本産 3 種カブリダニのミトコンドリア塩基配列の多様性

日本 典秀* (農業生物資源研究所, 〒 305-8634 茨城県つくば市大わし 1-2)・新宅 俊之・天野 洋 (千葉大学大学院・園芸学研究科・応用動物昆虫学研究室)

* Corresponding author: e-mail: hinomoto@affrc.go.jp

† Present address: Laboratory of Ecological Information, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

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1995, and since it was registered in 2003 it has been used widely for biological control of spider mites in greenhouses. Researchers initially suspected that the dramatic increase in numbers of the species, as mentioned above, was due to expansion of the distribution of this commercial strain. However, this increase in numbers preceded the importation of commercial strains (Amano et al., 2004), and the ecological traits were different among the commercial and field populations of *N. californicus* (Gotoh et al., 2005; Toyoshima and Hinomoto, 2004).

We attempted to determine the genetic background of *N. californicus* by analyzing the DNA sequences of the cytochrome *c* oxidase subunit I (COI) gene of mitochondrial DNA (mtDNA) to elucidate whether *N. californicus* is an invasive species. To compare the genetic diversity of this species with those of native and other cosmopolitan species, we included *N. womersleyi* (Schicha) and *Euseius finlandicus* (Oudemans) in our analysis.

MATERIALS AND METHODS

Mites

Six populations of *N. californicus*, eight of *N. womersleyi*, and four of *E. finlandicus* were used (Table 1). Until use, the two *Neoseiulus* species were kept under a 16-L 8-D photoperiod at $19\pm1^\circ\text{C}$ on detached bean leaves (*Phaseolus vulgaris* L.) placed on wet cotton. They were supplied with two-spotted spider mite, *Tetranychus urticae* Koch, as prey. In the case of *E. finlandicus*, collected females were used directly.

Table 1. Collection record of the phytoseiid mite populations used.

Species	Code	N*	Collection site	Plant	Date
<i>Neoseiulus californicus</i>	Nc1	3	Futtsu, Chiba	kudzu vine	May 2000
	Nc2	3	Ichihara, Chiba	Japanese pear	August 1995
	Nc3	3	Matsukawa, Nagano	pear	September 2000
	Nc4	3	Funao, Okayama	grape	November 2001
	Nc5	3	Yawatahama, Ehime	orange	July 2002
	Nc6	6	SPICAL® **		November 1995
<i>Neoseiulus womersleyi</i>	Nw1	3	Naganuma, Hokkaido	raspberry	August 1995
	Nw2	3	Assabu, Hokkaido	hog peanut	August 1998
	Nw3	3	Hirono, Fukushima	hog peanut	September 1999
	Nw4	3	Numazu, Shizuoka	kudzu vine	November 2000
	Nw5	3	Matsukawa, Nagano	Japanese pear	September 2000
	Nw6	3	Shinjo, Nara	chrysanthemum	September 1996
	Nw7	3	Setouchi, Kagoshima	harlequin glory bower	August 2001
	Nw8	3	Ishigaki, Okinawa	cucumber	September 1996
<i>Euseius finlandicus</i>	Ef1	3	Mutsu, Aomori	<i>Magnolia obovata</i>	August 2002
	Ef2	2	Tanakura, Fukushima	<i>Magnolia obovata</i>	August 2002
	Ef3	2	Hara, Nagano	<i>Magnolia obovata</i>	June 2002
	Ef4	2	Shirakawa, Gifu	<i>Magnolia obovata</i>	June 2002

* Number of individuals examined.

** SPICAL® is a commercial product (Arysta LifeScience, Japan).

DNA extraction, polymerase chain reaction, and sequencing

Two to six females from each population were analyzed. Total DNA was extracted from the whole body of each individual by using Genomic Prep Cells and a Tissue DNA Isolation Kit (GE Healthcare). It was then dissolved in 50 μ L of TE buffer (1 mM Tris and 0.1 mM EDTA). A fragment of the mitochondrial COI gene was amplified with the primers C1-J-1718 (5'-GGAGGATTTGGAATTGATTAGTTCC-3'; Simon et al., 1994) and NR (5'-TACAGCTCCTATAGATAAAAC-3'; Navajas et al., 1996). PCR amplification was performed in 30 μ L of reaction mixture containing 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.2 μ M of each primer, 1.2 μ L of DNA solution, 0.6 units of *TaKaRa Ex Taq*[®] DNA polymerase (Takara), and the buffer supplied with the polymerase. DNA amplification was performed in a 0.2-mL thin-walled PCR tube using a thermocycler (iCycler, Bio-Rad Laboratories), with the following profile: 94 °C for 3 min; 35 cycles of 94 °C for 1 min, 40 °C for 1 min, and 72 °C for 1 min; and 72 °C for 10 min.

Direct sequencing was conducted in both the forward and reverse directions by using the same primers as used for PCR. Sequencing reactions were performed in a total volume of 5 μ L. Each reaction mixture contained 0.5 μ L of the amplified fragment, which had been purified beforehand with a MinElute PCR Purification Kit (Qiagen) and dissolved in 10 μ L of distilled water, 1.65 pmol of primer, 1.0 μ L of BigDye Terminator Sequencing Reaction Mixture v. 3.1 (Applied Biosystems), and 0.5 μ L of 5X sequencing buffer (supplied with the BigDye kit). Cycle sequencing conditions were 30 cycles of 96 °C for 30 s, 50 °C for 15 s, and 60 °C for 4 min. The sequencing reaction products were purified by ethanol precipitation in accordance with the manufacturer's instructions. Sequencing was then performed with an automated ABI Prism[®] 3100 Genetic Analyzer (Applied Biosystems).

Sequence data analysis

After the sequences obtained had been aligned by using CLUSTAL W (Thompson et al., 1994), pairwise differences of each pair of sequences were also calculated by PAUP* version 4.0b10 (Swofford, 2002). The differences among three taxonomic levels (within population, within species, and between species) were analyzed, and the genetic compositions of the three species were compared. Statistical tests were conducted with R packages (R Development Core Team, 2008).

RESULTS AND DISCUSSION

The length of the amplified region of the COI gene, excluding primers, was 727 bp in *N. womersleyi* and *E. finlandicus* and 730 bp in *N. californicus*, which had one amino acid insertion compared with the other two species. The number of haplotypes found was 5 in *N. californicus*, 5 in *N. womersleyi*, and 6 in *E. finlandicus* (Tables 2 to 4). The sequence data obtained were deposited in the DDBJ databases (accession numbers AB500127–AB500142). The average proportion of base difference among haplotypes within species also differed significantly among the three species (all $P < 0.05$; Kruskal-Wallis rank sum test followed by pairwise comparisons using Wilcoxon rank sum test adjusted by Holm's method); it was 0.0030 ± 0.0016 in *N. californicus*, 0.0359 ± 0.0239 in *N. womersleyi*, and 0.0775 ± 0.0516 in *E. finlandicus*. The proportion of base difference among species was 0.2755 ± 0.0132 , which was significantly higher

Table 2. Numbers of individuals for each COI haplotype in each population of *Neoseiulus californicus*.

Haplotype	Population*						Accession no.
	Nc1	Nc2	Nc3	Nc4	Nc5	Nc6	
C1	3	1	2	2	1		AB500127
C2						3	AB500128
C3		2	1		1	3	AB500129
C4				1			AB500130
C5					1		AB500131

* Codes for each population are shown in Table 1.

Table 3. Numbers of individuals for each COI haplotype in each population of *Neoseiulus womersleyi*.

Haplotype	Population*								Accession no.
	Nw1	Nw2	Nw3	Nw4	Nw5	Nw6	Nw7	Nw8	
W1			3	3	3	3			AB500132
W2							3		AB500133
W3								3	AB500134
W4		3							AB500135
W5	3								AB500136

* Codes for each population are shown in Table 1.

Table 4. Numbers of individuals for each COI haplotype in each population of *Euseius finlandicus*.

Haplotype	Population*				Accession no.
	Ef1	Ef2	Ef3	Ef4	
F1	2				AB500137
F2			2		AB500138
F3	1				AB500139
F4		1			AB500140
F5		1			AB500141
F6				2	AB500142

* Codes for each population are shown in Table 1.

than the intraspecific differences ($P < 0.001$; Wilcoxon rank sum test).

Neoseiulus womersleyi used to have a dominant position in the phytoseiid fauna throughout Japan. However, in the 1990s *N. californicus* replaced *N. womersleyi* in many orchards (Amano et al., 2004). This change in species composition has been investigated from various viewpoints (Amano et al., 2004; Gotoh et al., 2005), but the reason behind it is still not clear. In recent years, *N. californicus* has been observed commonly in the Kyushu area (Kishimoto et al., 2007), suggesting that its distributional range is still expanding. The recent geographical expansion of the distribution of *N. californicus* and the coincident dramatic disappearance of *N. womersleyi* seem to represent the typical competitive exclusion of a native species by an invasive one.

Usually, invasive species show low levels of genetic diversity because of population

bottlenecks (Davies et al., 1999; Sakai et al., 2001). The low level of mtDNA diversity found in *N. californicus* therefore suggests recent invasion by this species. Furthermore, *N. californicus* showed common haplotypes (C1) among field populations while other two species had area-specific haplotypes. These results, together with those of the previous observation, suggest that *N. californicus* invaded Japan only recently. The results also showed that not all field populations descended from the commercial strain, because haplotypes found in field populations were different from those of the commercial strain (Table 2). Spider mites, prey of phytoseiid mites, are often caught during inspections by Japanese plant quarantine services (e.g., Ehara and Masaki, 2001). Phytoseiid mites usually show a close association with spider mites, and it is likely that these predators also invaded Japan through human activity. *Neoseiulus californicus* was most probably one of these species and replaced the niche occupied by *N. womersleyi*, although further evidence is needed to confirm this hypothesis.

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