Molecular phylogenetic relationship of *Stigmaeopsis* spider mites (Acari: Tetranychidae) collected from Yamagata Prefecture

Katsura Ito[1,*], Nanako Yokoyama[1], Hiroshi Hayakawa[2], Yukio Minamiya[2], Jun Yokoyama[3] and Tatsuya Fukuda[1]

山形大学紀要（自然科学）第17巻第２号別刷
平成23年（2011） ２月

Bull. of Yamagata Univ., Nat Sci., Vol.17, No. 2, Feb. 2011

Molecular phylogenetic relationship of *Stigmaeopsis* spider mites (Acari: Tetranychidae) collected from Yamagata Prefecture

Katsura Ito[1, *], Nanako Yokoyama[1], Hiroshi Hayakawa[2], Yukio Minamiya[2], Jun Yokoyama[3] and Tatsuya Fukuda[1]

(Received March 1, 2010)

Abstract

We investigated the phylogenetic relationships of *Stigmaeopsis* spider mites (Acari: Tetranychidae) using sequence variation in the cytochrome oxidase subunit I (COI) region of mitochondria DNA. We sequenced a total of 32 samples including four samples of *S. takahashii* and ten of *S. longus* from Yamagata Prefecture. Phylogenetic analyses based on the neighbor-joining and the Bayesian method revealed that *Stigmaeopsis* consists of five monophyletic groups corresponding to each species. Among them, the monophyletic group of *S. longus* was further divided into two subgroups; one is the northern group composed of the samples from the Hokkaido and Tohoku districts, and the other is the southern group of the samples from the Tohoku, Hokuriku, Shikoku, and Kyushu districts. This suggests that the current distribution of this species has employed two routes of expansion. It is interesting that the samples of *S. longus* from Yamagata Prefecture were divided into the two groups. This implies that *S. longus* had multi-expanded patterns of distribution in this prefecture.

Introduction

Spider mites (Acari: Tetranychidae) usually live on the underside of various plant leaves and suck fluid from mesophyll tissue. Among them, mites of the genus *Stigmaeopsis* are characterized by their curious life styles. They weave dense silken

[1]: Faculty of Agriculture, Kochi University, 200 Monobeotsu, Nankoku, Kochi 783-8502, Japan
[2]: The United Graduate School of Agricultural Science, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan
[3]: Faculty of Science, Yamagata University, 1-4-12 Kojirakawa-machi, Yamagata 990-8560, Japan
[*]: corresponding author
Katsura Ito
Faculty of Agriculture, Kochi University, 200 Monobeotsu, Nankoku, Kochi 783-8502, Japan
E-mail: ktr@kochi-u.ac.jp
Tel & Fax: +81-88-864-5136

nests along the midrib or the edge of leaves of bamboos (*Sasa* spp., *Pleioblastus* spp. etc., Poaceae; Bambusoideae) or Chinese silver grass (*Miscanthus sinensis*, Poaceae; Panicoideae). They feed and reproduce within the nests, causing white stippling throughout the leaf surface. To date, five species have been reported to occur in Japan (Saito et al., 2004). Among them, *S. saharai*, *S. takahashii*, *S. celarius*, and *S. longus* utilize the leaves of bamboo species, whereas *S. miscanthi*, which comprises two ecologically different forms (LW and HG types: Saito, 1995; Saito and Sahara, 1999), is restricted to the leaves of silver grass.

The classification of *Stigmaeopsis* species has long been confounded because of their similarity in ecological and morphological attributes (reviewed in Saito et al., 2004). A recent molecular phylogenetic study using the partial sequences of the cytochrome oxidase subunit I (COI) on mitochondria DNA (mtDNA) indicated that each form of *S. miscanthi* corresponds to a separate monophyletic group, but bamboo-inhabiting species comprise paraphyletic assemblages (Ito and Fukuda, 2009). Although this phylogenetic analysis was based on as many as 81 *Stigmaeopsis* samples from a wide area of Japan, samples from the Tohoku district of Honshu was completely absent therein. To clarify the detailed phylogenetic relationships of *Stigmaeopsis* spider mites in Japan, analyzing samples of this area is necessary.

Yamagata Prefecture locates in the Tohoku district, northern Honshu of Japan, faces the Sea of Japan in the western area, and has numerous peaks approx. 2,000 m in elevation such as Mts. Gassan and Chokai. Thus, this area may exhibit climatic variation associated with altitudinal gradient. In addition, there are many species of dwarf bamboo of the genus *Sasa* (about 20 species, Yuki, 1992), on which *S. takahashii* and *S. longus* are frequently found. The performance of *Stigmaeopsis* mites is considered quite different among *Sasa* species (Y. Saito, pers. comm.). Therefore, this area is considered heterogeneous in both biotic and abiotic environments for these species, so that differentiation of strains may be occurring through adaptation to different conditions. Here, to better understand the phylogenetic relationship of *Stigmaeopsis* spider mites in Japan, we analyzed the polymorphism in the COI sequences of these species sampled in this prefecture, and determined their phylogenetic positions within the genus.

Materials and Methods

Mites

Fourteen populations (four of *S. takahashii* and ten of *S. longus*) representing ten localities were collected from the Yamagata Prefecture in September, 2009 (Table 1). Other populations were obtained in 2007-2008. Part of DNA samples were identical with those used in Ito and Fukuda (2009), and were reanalyzed for

Table 1 Summary of spider mites analyzed in this study

Label	Host plant	Species	Locality			Sampling date	Accession No.	Sites analyzed
			District	Prefecture	City			
Muroto	*Miscanthus sinensis*	*S. miscanthi* (HG)	Shikoku	Kochi	Muroto	27 May, 2007	AB429416	47 - 519 , 694 - 1003
Warabino	*Miscanthus sinensis*	*S. miscanthi* (LW)	Shikoku	Kochi	Kami	5 June, 2007	AB429422	57 - 529 , 704 - 1013
Suita	*Phyllostachys heterocycla*	*S. celarius*	Kinki	Osaka	Suita	29 May, 2007	AB429469	1 - 473 , 648 - 957
Monobe	*Pleioblastus simonii*	*S. celarius*	Shikoku	Kochi	Nankoku	29 Feb., 2008	AB531823	56 - 528 , 703 - 1012
Tsukuba	*Pleioblastus chino*	*S. celarius*	Kanto	Ibaraki	Tsukuba	17 Mar., 2009	AB531824	29 - 501 , 676 - 985
Oyafuru1	*Sasa senanensis*	*S. longus*	Hokkaido	Hokkaido	Ishikari	18 June, 2007	AB429467	59 - 531, 706 - 1015
Sikotuko	*Sasa sp.*	*S. longus*	Hokkaido	Hokkaido	Chitose	13 Aug., 2007	AB429465	44 - 516, 691 - 1000
Oisida_Our	*Sasa kurilensis*	*S. longus*	Tohoku	Yamagata	Oisidamati	23 Sep., 2009	AB531825	29 - 501, 676 - 985
Oisida_Kaw	*Sasa kurilensis*	*S. longus*	Tohoku	Yamagata	Oisidamati	23 Sep., 2009	AB531826	29 - 501, 676 - 985
Kaminoyama_Mae1	*Sasa kurilensis*	*S. longus*	Tohoku	Yamagata	Kaminoyama	22 Sep., 2009	AB531827	29 - 501, 676 - 985
Kaminoyama_Kab	*Sasa palmata*	*S. longus*	Tohoku	Yamagata	Kaminoyama	22 Sep., 2009	AB531828	29 - 501, 676 - 985
Kaminoyama_Nak	*Sasa palmata*	*S. longus*	Tohoku	Yamagata	Kaminoyama	22 Sep., 2009	AB531829	29 - 501, 676 - 985
Nisikawa_Yum	*Sasa kurilensis*	*S. longus*	Tohoku	Yamagata	Nisikawamati	23 Sep., 2009	AB531830	29 - 501, 676 - 985
Nisikawa_Siz	*Sasa kurilensis*	*S. longus*	Tohoku	Yamagata	Nisikawamati	23 Sep., 2009	AB531831	29 - 501, 676 - 985
Yamagata_Kok1	*Sasa senanensis*	*S. longus*	Tohoku	Yamagata	Yamagata	22 Sep., 2009	AB531832	29 - 501, 676 - 985
Takahata_Nii1	*Sasa kurilensis*	*S. longus*	Tohoku	Yamagata	Takahatamati	22 Sep., 2009	AB531833	29 - 501, 676 - 985
Nanyo_Man	*Sasamorpha borealis*	*S. longus*	Tohoku	Yamagata	Nan'yo	22 Sep., 2009	AB531834	29 - 501, 676 - 985
Tsunagi1	*Sasa kurilensis*	*S. longus*	Tohoku	Iwate	Morioka	16 Mar., 2009	AB531835	44 - 516 , 691 - 1000
Arisoumi	*Sasa sp.*	*S. longus*	Hokuriku	Toyama	Namerikawa	11 Sep., 2007	AB429485	53 - 525 , 700 - 1009
Shoumyou	*Sasa sp.*	*S. longus*	Hokuriku	Toyama	Tateyama	12 Sep., 2007	AB429483	1 - 473 , 648 - 957
Hitsuzan2	*Sasa oshidensis*	*S. longus*	Shikoku	Kochi	Kochi	30 Aug., 2007	AB429479	5 - 477 , 652 - 961
Kikuchi_1	*Sasa sp.*	*S. longus*	Kyushu	Kumamoto	Kikuchi	22 July, 2007	AB429450	44 - 516 , 691 - 1000
Kikuchi_2	*Sasa sp.*	*S. longus*	Kyushu	Kumamoto	Kikuchi	22 July, 2007	AB429449	44 - 516 , 691 - 1000
Asiribetu	*Sasa kurilensis*	*S. saharai*	Hokkaido	Hokkaido	Sapporo	18 June, 2007	AB429466	1 - 473 , 648 - 957
Oyafuru2	*Sasa senanensis*	*S. takahashii*	Hokkaido	Hokkaido	Oyafuru	18 June, 2007	AB429468	17 - 489 , 664 - 973
Yamagata_Kok2	*Sasa senanensis*	*S. takahashii*	Tohoku	Yamagata	Yamagata	22 Sep., 2009	AB531836	29 - 501, 676 - 985
Yonezawa_Kar	*Sasa palmata*	*S. takahashii*	Tohoku	Yamagata	Yonezawa	22 Sep., 2009	AB531837	29 - 501, 676 - 985
Takahata_Nii2	*Sasa kurilensis*	*S. takahashii*	Tohoku	Yamagata	Takahatamati	22 Sep., 2009	AB531838	29 - 501, 676 - 985
Kaminoyama_Mae2	*Sasa kurilensis*	*S. takahashii*	Tohoku	Yamagata	Kaminoyama	22 Sep., 2009	AB531839	29 - 501, 676 - 985
Tsunagi2	*Sasa palmata*	*S. takahashii*	Tohoku	Iwate	Morioka	16 Mar., 2009	AB531840	29 - 501, 676 - 985
Tutigoya	*Sasa sp.*	*S. takahashii*	Shikoku	Ehime	Kumakogentyo	7 Sep., 2007	AB429481	47 - 519 , 694 - 1003
Marutaki	*Sasa sp.*	*S. takahashii*	Shikoku	Ehime	Kumakogentyo	7 Sep., 2007	AB429482	29 - 501, 676 - 985
Outgroup	*Castanopsis cuspodata*	*Eotetranychus shii*	Shikoku	Kochi	Konan	17 Dec., 2007	AB531841	47 - 519 , 694 - 1003

the additional sequences of the COI region for this study (EMBL/GenBank/DDBJ accession number: AB429416－429485, Table 1). Mite species was identified based on the relative length of female dorsal setae on mounted specimen by microscopy (Saito et al., 2004; Ito and Fukuda, 2009). *Eotetranychus shii* (Acari: Tetranychidae), which parasitizes *Castanopsis cuspidata* (Fagaceae), was used as an outgroup taxon for the phylogenetic analysis (AB531841).

DNA extraction and sequencing analyses

The whole body of a single individual from each population was crushed in a 1.5 mL microcentrifuge tube containing 200 μL of HMW buffer (10 mM Tris, 150 mM NaCl, 10 mM ethylenediaminetetraacetic acid (EDTA)-2Na (pH 8.0), 1.255% (w/v) sodium dodecylsulfate (SDS) and 0.1 mg/mL proteinase K). The mixture was incubated at 55°C for 30 min. After incubation, the mixture was directly

precipitated in ethanol, and DNA was collected through centrifugation. Pellets of DNA were resuspended in 20 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) (Ito and Fukuda (2009) for detailed procedure).

The following pairs of degenerate primers were used for PCR amplification of the partial COI region (each franks approx. 500 bp): Orank1F: 5'-CTG GWT TRA TRG GKA CYT CT-3' (forward primer) and Tosa2R: 5'-CHC CWG CTA AWA CWG GTA AW-3' (reverse primer) for the former region; Ito2F: 5'-CWA TTG CTA GYT CWA TTA AT-3' (forward) and KatsuraR: 5'-ATR TAR TAW GWR TCA TGT AA-3' (reverse) for the latter region. They were also used as the sequencing primers. PCR was performed in a 50 μl reaction mixture containing 1.25 μl of the DNA sample, 1x PCR buffer (10 mM Tris-HCl buffer (pH 8.3 at 25°C), 50 mM KCl, and 1.5 mM $MgCl_2$), 0.16 mM of each dNTP, 0.3 mM of each primer, and 1.25 U of rTaq DNA polymerase (TOYOBO). The program consisted of an initial denaturation step at 94°C for 30 sec, 45 cycles of incubation steps at 94°C for 1 min, 48°C for 2 min, and 72°C for 2 min, with a final extension step at 72°C for 15 min. The resultant mixtures were subjected to electrophoresis in 1% low-melting-temperature agarose gels to purify amplified products. The DNA bands were cut out with razor blades and purified using the Gel Extraction Kit (Qiagen) or the ordinary phenol-chloroform method. These products were sequenced using BigDye Terminator v3.1 (Applied Biosystems) on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. All sequence data have been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases (Table 1).

Date analyses

To construct a phylogenetic tree based on the COI sequences of *Stigmaeopsis* spider mites and its allied species, the sequences of successfully amplified regions were aligned using Clustal W (Thompson et al., 1994). For several samples, either end or intermediate part of the sequence suffered from a high frequency of uncertain bases, so that these sites were excluded from the analyses. The sites analyzed in each sample were summarized in Table 1.

The phylogenetic tree were constructed using the neighbor-joining (NJ) method with MEGA4 (Tamura et al., 2007) including all codon positions. The evolutionary distances were computed using the maximum composite likelihood method based on Tamura-Nei distance, which assumes the equality of substitution pattern among lineages and of substitution rates among sites (Tamura et al., 2004, 2007). The reliability of branching patterns was assessed by 1,000 bootstrap resamplings.

Phylogenetic analysis was also performed under Bayesian inference with

MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck, 2003). In the analysis, we adopted general time reversal (GTR) model, which is frequently applied for coding sequences. Partition was specified so that each codon position was assigned to a different substitution rate (nst = 6, ratepr = variable). Four independent chains were run at the same time. The temperature for heating the chains was 0.2 as default. Trials were run for 1,000,000 generations, and trees were retained every 100 generations. The first 2,500 trees before convergence were discarded as burn-in.

Results

The total length of the sequences used for the phylogenetic analysis was 783 bp. Base deletion was not found in any sample. In the NJ tree, *Stigmaeopsis* spider mites consisted of five monophyletic groups which correspond to each species; *S. saharai*, *S. takahashii*, *S. celarius*, *S. longus*, and *S. miscanthi* (Fig. 1). All four

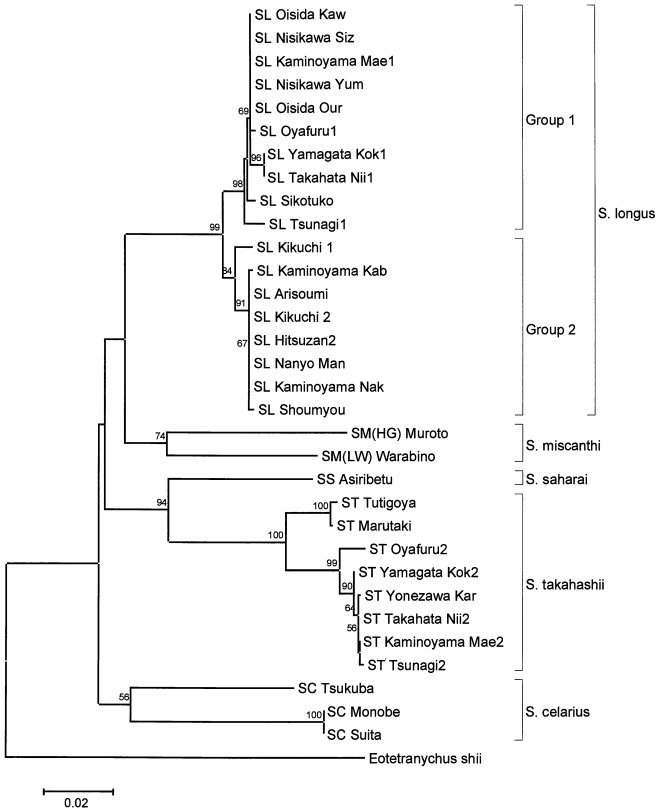

Fig. 1. Phylogenetic tree of *Stigmaeopsis* spider mites using the neighbor-joining method: SS, *S. saharai*; ST, *S. takahashii*, SC, *S. celarius*; SL, *S. longus*; SM (LW), *S. miscanthi* (LW); SM (HG), *S. miscanthi* (HG). The numbers beside branches indicate the bootstrap value (>50%). For other abbreviations, see Table 1.

samples of *S. takahashii* from Yamagata Prefecture belonged to the monophyletic group representing this species with the other *S. takahashii* samples from different areas. This group was strongly supported by very high bootstrap value (100%), and was sister to the sample of *S. saharai* from Hokkaido Prefecture. Two *S. takahashii* samples from the mountainous regions of the Shikoku district (Tutigoya and Marutaki) formed a distinct subgroup within this group (100%).

Ten samples of *S. longus* from Yamagata Prefecture were divided into two subgroups (groups 1 and 2) within the monophyletic group representing this species. Seven samples of *S. longus* collected from Oisida, Nisikawa, Kaminoyama, Yamagata and Takahata were included in group 1 and three from Kaminoyama and Nanyo were involved in group 2. Bootstrap values of each group were very high

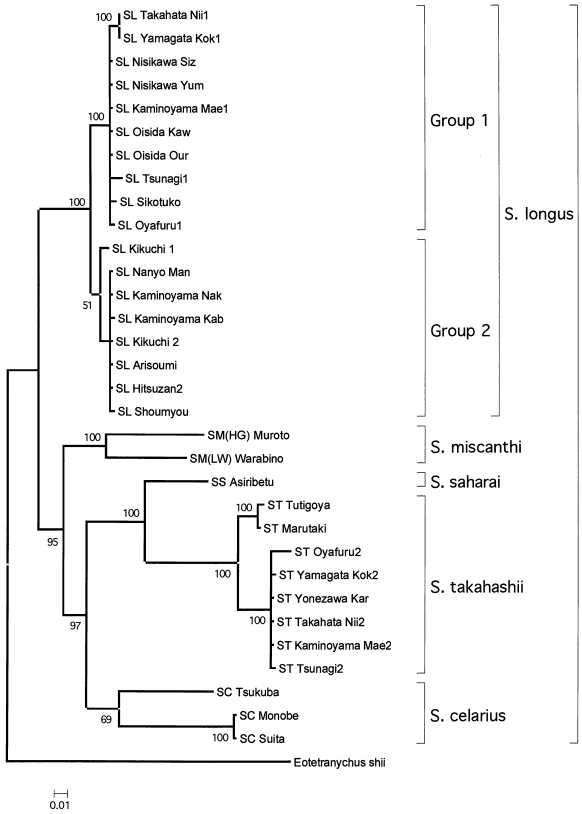

Fig. 2. Phylogenetic tree of *Stigmaeopsis* spider mites inferred from the Bayesian method. See legend to Fig. 1 for explanation of taxa. The numbers beside branches indicate posterior probabilities. For other abbreviations, see Table 1.

(group 1: 98%; group 2: 84%). The former group also contained the samples from the northern part of Japan (the Hokkaido and Tohoku districts), whereas the latter is composed of those of southern part of Japan (the Tohoku, Hokuriku, Shikoku, and Kyushu districts) (Fig. 3). The monophyletic group of *S. longus* was sister to that of *S. miscanthi* mites, which live on the leaves of *M. sinensis*.

The Bayesian tree showed almost the same shape as the NJ one, except that the clade of *S. miscanthi* is not a sister to *S. longus* but to the clade composed of other three bamboo-inhabiting species (Fig. 2).

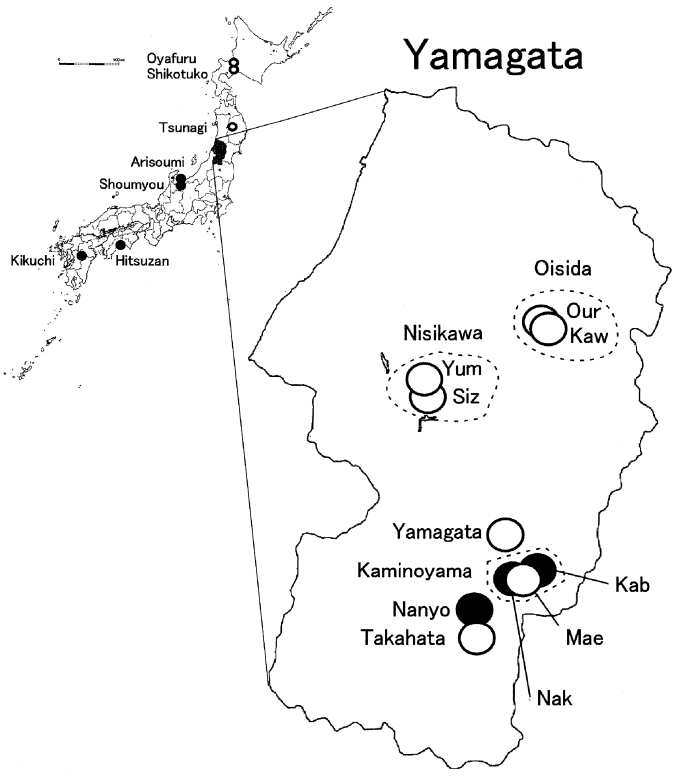

Fig. 3. Geographical distribution of two lineages of *Stigmaeopsis longus*. ○ , group 1 (northern group); ● , group 2 (southern group). See Table 1 for abbreviations.

Discussion

Till now, molecular phylogenetic studies using sequence variation in gene regions on mtDNA are increasing in taxonomic and biogeographical researches (Avise, 2000), and continually improving the understanding of biological diversity of spider mites (e.g., Ros and Breeuwer, 2007; Jeyaprakash and Hoy, 2009). The previous study of *Stigmaeopsis* phylogeny based on part of mtDNA sequences suggest that morphological classification of this genus does not reflect phylogenetic relationships (Ito and Fukuda, 2009). However, because the previous study lacked the samples of bamboo-inhabiting mites from the Tohoku district that is rich in *Sasa* species, phylogenetic relationship of mites strongly associated with *Sasa* species (*S. saharai, S. takahashii,* and *S. longus*) was not sufficiently elucidated. Many samples of *S. longus* in Yamagata Prefecture were added in the present study. We discuss infraspecific divergence in the following part.

In the present study, the samples of *S. longus* in Yamagata Prefecture and other areas constitute a monophyletic group, and the group was further divided into two subgroups (groups 1 and 2, Figs. 1 and 2). The samples of *S. longus* from Sikotuko and Oyafuru of Hokkaido Prefecture and Tsunagi of Iwate Prefecture were involved in group 1, and the samples from Arisoumi and Shoumyou of Toyama Prefecture, Hitsuzan of Kochi Prefecture, and Kikuchi of Kumamoto Prefecture were included in group 2 (Fig. 1). These results suggest that *S. longus* is composed of two genetically distinct groups; one is distributed from the Hokkaido district to Yamagata Prefecture, and the other is from Yamagata Prefecture to the Kyushu district. Furthermore, it is very interesting that both groups sympatrically occur in the southern part of Yamagata Prefecture (Fig. 3). Although our study could not indicate the direction of the range expansion within each group, this suggests that the current distribution in this species is a result of separate routes of range expansion of formerly differentiated subgroups.

At present, factors causing differentiation within *S. longus* are not known. In general, speciation of spider mites is closely related with predators and host plants (reviewed in Helle and Sabelis, 1985). Nest size variation, and eventually speciation, among *Stigmaeopsis* species is postulated to reflect antipredatory strategies: a large nest constructed by *S. longus* affords many mites inside and they can counterattack against intruding larvae of predatory mites, whereas a small nest by *S. takahashii* and *S. saharai* precludes invasion of large-body predators or lowers the efficiency of predation through scattering nests over host leaves (Saito, 2010). However, cryptic strains in *S. longus* found in this study appear not to vary in nest sizes and related morphological characters (lengths of dorsal setae). Therefore, adaptation to different host plants rather than predation might have played an important role in differentiation. Notably, there appears to be variation

among populations for host-plant availability even within *Stigmaeopsis* species (K. Ito, unpublished; Y. Saito, pers. comm.). Thus, availability of host plants in populations of these strains should be further investigated to infer the importance of host plants in speciation of *Stigmaeopsis* mites.

The phylogenetic topology obtained here is consistent with that of preceding study employing 28S ribosomal DNA (Sakagami et al., 2009) in three points. First, two forms (HG and LW) of *S. miscanthi* are monophyletic. Second, two forms of *S. miscanthi* and *S. longus* are monophyletic. Finally, *S. saharai* and a population of *S. takahashii* (Oyafuru) are monophyletic, though the other population of *S. takahashii* (Sapporo) employed in their study is positioned in the clade of (*S. celarius, S. longus,* and *S. miscanthi*) (this population is not analyzed here). The present results incorporating *S. takahashii* samples from a broad area of Japan support that *S. saharai* and *S. takahashii* are phylogenetically closely related. On the other hand, the position of *S. celarius* appears to be quite different. In their study, the two populations of *S. celarius* is positioned in the clade with *S. longus*, *S. miscanthi*, and *S. takahashii* (Sapporo), whereas the three populations of *S. celarius* analyzed here are a sister to the rest. However, in the preliminary test, a subtle change of length of sequences analyzed altered the estimated phylogenetic position of *S. celarius* (results not shown). Therefore, phylogenetic position of this species should be further scrutinized.

In general, a morphological species is often a composite of cryptic species, which cannot easily be distinguished from each other using traditional systematic methods (King, 1993). Molecular data are common sources of additional markers in such cases, as they are numerous, easy to obtain, and bridge the gap between intra- and interspecific variation (Templeton, 2001). Avise et al. (1987) claimed that mtDNA sequences are particularly useful in investigating inter- and intraspecific variation because they evolve rapidly, are maternally inherited and effectively haploid, have limited recombination, and are robust against degradation. For example, Kawazoe et al. (2008) found that morphological species of *Sennertia* mites (Acari: Chaetodactylidae), which reproduce in the nests of long-tongued bees or carpenter bees, include cryptic species that are differentiated in host association using the phylogenetic analyses of the COI region and the amplified fragment length polymorphism (AFLP). In the present study, *S. longus* showed nucleotide differentiation between the subgroups. To investigate whether these subgroups are established as biological species, we should investigate the host ranges of each group as well as reproductive isolation between them as conducted in defining the current four species (Saito and Takahashi, 1982; Mori, 2000).

In summary, our analyses indicate the geographic differentiation of *S. longus* in Yamagata Prefecture. This differentiation may have been caused in association with the geographical and host-plant features of Japan. Further phylogenetic

researches on *Stigmaeopsis* spider mites incorporating samples from a wide area of Japan are needed to elucidate how the geographical and host-plant features have affected their diversification.

Acknowledgements

We wish to thank Mr. K. Ohga and Ms. K. Matsuyama for much help on experiments and sample collection. We thank Drs. Y. Saito, K. Sahara, and T. Sakagami for helpful criticisms on this study and kindly providing materials. This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (to J.Y. and T.F.).

References

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A. and Saunders, N.C. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics, Annu. Rev. Ecol. Syst., 18, 489-522, 1987.

Avise, J.C. Phylogeography, Harvard University Press, Cambridge, Massachusetts, London, 2000.

Helle, W. and Sabelis M.W. Spider mites. Their biology, natural enemies and control 1A, Elsevier, Amsterdam, 1985.

Ito, K. and Fukuda, T. Molecular phylogeny of *Stigmaeopsis* spider mites based on the Cytochrome Oxidase subunit I (COI) region of mitochondria DNA, Appl. Entomol. Zool., 44, 343-355, 2009.

Jeyaprakash, A. and Hoy, M. First divergence time estimate of spiders, scorpions, mites and ticks (subphylum: Chelicerata) inferred from mitochondrial phylogeny, Exp. Appl. Acarol., 47, 1-18, 2009.

Kawazoe, K., Kawakita, A., Kameda, Y. and Kato, M. Redundant species, cryptic host-associated divergence, and secondary shift in *Sennertia* mites (Acari: Chaetodactylidae) associated with four large carpenter bees (Hymenoptera: Apidae: *Xylocopa*) in the Japanese island arc, Mol. Phylogenet. Evol., 49, 503-513, 2008.

King, M. Species evolution: the role of chromosome change, Cambridge University Press, Melbourne, 1993.

Mori, K. Factors causing variation in social systems of spider mites, *Schizotetranychus celarius* species group (Acari: Tetranychidae). PhD Thesis, Hokkaido University, 2000.

Ronquist, F. and Huelsenbeck J.P. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19, 1572-1574, 2003.

Ros, V. and Breeuwer, J. Spider mite (Acari: Tetranychidae) mitochondrial COI phylogeny reviewed: host plant relationships, phylogeography, reproductive parasites and barcoding. Exp. Appl. Acarol., 42, 239-262, 2007.

Saito, Y. Clinal variation in male-to-male antagonism and weaponry in a subsocial mite. Evolution, 49, 413-417, 1995.

Saito, Y. Plant mites and sociality, Springer, Tokyo, 2010.

Saito, Y. and Sahara, K. Two clinal trends in male-male aggressiveness in a subsocial spider mite. Behav. Ecol. Sociobiol., 46, 25-29, 1999.

Saito, Y. and Takahashi, K. Study on variation of *Schizotetranychus celarius* (Banks) II. Comparison of mode of life between two sympatric forms (Acarina: Tetranychidae). Jpn. J. Ecol., 32, 69-78, 1982 (in Japanese).

Saito, Y., Mori, K., Sakagami, T. and Lin, J. Reinstatement of the genus *Stigmaeopsis* Banks, with descriptions of two new species (Acari, Tetranychidae). Ann. Entomol. Soc. Am., 97, 635-646, 2004.

Sakagami, T., Saito, Y., Kongchuensin, M. and Sahara, K. Molecular phylogeny of *Stigmaeopsis*, with special reference to speciation through host plant shift. Ann. Entomol. Soc. Am., 102, 360-366, 2009.

Tamura, K., Nei, M. and Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. USA., 101, 11030-11035, 2004.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol., 24, 1596-1599, 2007.

Templeton, A.R. Using phylogeographic analyses of gene trees to test species status and processes. Mol. Ecol., 10, 779－791, 2001.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. 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, 1994.

Yuki, Y. New edition of the Flora of Yamagata Prefecture, The publication committee of the New edition of the Flora of Yamagata Prefecture, Yamagata, 1992 (in Japanese).