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Population structure of the predatory mite *Neoseiulus womersleyi* in a tea field based on an analysis of microsatellite DNA markers

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Abstract The predatory mite *Neoseiulus womersleyi* (Schicha) (Acari: Phytoseiidae) is an important natural enemy of the Kanzawa spider mite, *Tetranychus kanzawaki* Kishida (Acari: Tetranychidae), in tea fields. Attraction and preservation of natural enemies by habitat management to reduce the need for acaricide sprays is thought to enhance the activity of *N. womersleyi*. To better conserve *N. womersleyi* in the field, however, it is essential to elucidate the population genetic structure of this species. To this end, we developed ten microsatellite DNA markers for *N. womersleyi*. We then evaluated population structure of *N. womersleyi* collected from a tea field, where Mexican sunflower, *Tithonia rotundifolia* (Mill.), was planted to preserve *N. womersleyi*. Seventy-seven adult females were collected from four sites within 200 m. The fixation indexes F_{ST} among subpopulations were not significantly different. The kinship coefficients between individuals did not differ significantly within a site as a function of the sampling dates, but the coefficients gradually decreased with increasing distance. Bayesian clustering analysis revealed that the population consisted of three genetic clusters, and that subpopulations within 100 m, including those collected on *T. rotundifolia*, were genetically similar to each other. Given the previously observed population dynamics of *N. womersleyi*, it appears that the area inhabited by a given cluster of the mite did not exceed 100 m. The estimation of population structure using microsatellite markers will provide valuable information in conservation biological control.

Keywords *Neoseiulus womersleyi* · Conservation biological control · Natural enemy · Genetic markers · Dispersal · Kinship coefficients

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Introduction

Commercial agricultural fields are regularly disturbed by farming activities such as plowing, planting and harvesting (e.g. Landis et al. 2000; Lee et al. 2001; Lester et al. 1998). Pesticide and herbicide sprays are one of the most dramatic disturbances that affect the organisms that inhabit these fields, and it has been impossible to completely eliminate these sprays in commercial farming because many pest species can seriously injure agricultural crops in the absence of this protection. Unfortunately, pesticide sprays also damage the natural enemies of arthropod pests (Hassan et al. 1987); as a result, chemical control and biological control have been largely incompatible. However, the use of selective pesticides, which selectively kill a target pest species and have a reduced impact on other organisms, has grown in recent decades and increased usage of these agrichemicals should facilitate the integration of biological control within integrated pest management systems (Naranjo 2001).

Conservation biological control is the practice of enhancing the efficacy of natural enemies through modification of the environment or of existing pesticide practices (Eilenberg et al. 2001). In this approach, the attraction and the preservation of natural enemies by means of habitat management enhance the activity of these organisms. Planning for crop-plant diversity benefits the biological control of pest arthropods (Pimentel 2008). However, to better conserve the natural enemies of agricultural pests, it is necessary to consider the spatial and temporal changes in their distribution and movement patterns in the field.

In commercial tea (*Camellia sinensis* (L.)) fields, the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae), has been one of the most important pests. This mite has developed a high degree of resistance to various acaricides (Aiki et al. 2005; Goka 1998; Kuwahara 1982, 1984; Kuwahara et al. 1982; Mizutani et al. 1988; Osakabe 1968), so it has become increasingly important to find ways to control the species using natural enemies. The phytoseiid mite *Neoseiulus womersleyi* (Schicha) (Acari: Phytoseiidae) is one of the most important predators of the Kanzawa spider mite (Hamamura 1986), and some *N. womersleyi* strains resistant to pesticides have been found (Hamamura 1986; Mochizuki 1990, 1994). Thus, the species is expected to potentially control *T. kanzawai* even where pesticides were used to control other pest species.

Recently Todokoro and Isobe (2010) found that Mexican sunflower, *Tithonia rotundifolia* (Mill.), was effective at preserving *N. womersleyi* in tea fields. They planted Mexican sunflowers that had been artificially infested with *Tetranychus urticae* Koch besides the ridges of tea plants. Indigenous *N. womersleyi* then fed on the *T. urticae* and their populations naturally increased on the plants; thereafter, the predatory mites moved to the tea plants and began to control *T. kanzawai* populations on these plants. *Tetranychus urticae* does not injure tea plants, thus the combination of *T. rotundifolia* and *T. urticae* leads to a rapid increase of the *N. womersleyi* population. If recognizing their distribution, origin, and movement in the field, appropriate planting of *T. rotundifolia* in geographic scale can be conducted. Unfortunately, it is practically very difficult to directly and continuously observe the dispersal of small organisms such as mites over generations, although efforts to estimate thier movements have been attempted (e.g. Barbar et al. 2006; Hoy et al. 1985; Tixier et al. 1998, 2000). As an alternative, the estimation of gene flow using genetic markers would provide insights into the mite's population structure and dispersal patterns.

Microsatellites, which are short stretches of tandem-repeated sequences of one to five nucleotides, are ubiquitous in eukaryotic genomes and are highly polymorphic (Hancock 1999). Their high levels of polymorphism make them a suitable marker for studying intra- and interpopulation variation. Recently, microsatellite markers have also been developed for several mite species (e.g. Bailly et al. 2004; Navajas et al. 1998; Nishimura et al. 2003; Osakabe et al. 2000; Uesugi and Osakabe 2007). However, these markers have not been applied to phytoseiid mites in the context of conservation biological control. In the present study, we developed microsatellite DNA markers for *N. womersleyi*, and used this tool to investigate the population genetic structure of the mite in an experimental tea field.

Materials and methods

Isolation of microsatellite loci

We constructed a microsatellite-enriched library for *N. womersleyi* according to the method described by Schlötterer (1998), with some modifications. For isolation of the microsatellite loci, we used a laboratory strain collected in Morioka, Iwate, Japan (39.768°N, 141.135°E; Toyoshima and Hinomoto 2003). Genomic DNA was extracted from the whole body of 20 adult females using a Wizard® Genomic DNA Purification Kit (Promega). Mites were placed in a 1.5-mL microtube and crushed using several zirconium dioxide beads (1.5 mm in diameter) in 50 μL of the supplied nuclei lysis solution by an bead mill (Shakemaster®; Bio Medical Science) for 5 min. The DNA was extracted according to the manufacturer's instructions, then was dissolved in 50 μL of TE buffer (1 mM Tris, 0.1 mM EDTA).

The DNA was digested with the restriction enzymes *Nhe*I and either *Alu*I, *Csp*45I, *Mbo*I, or *Rsa*I. Digested DNA fragments were ligated overnight with SNX linker (forward, 5′-CTAAGGCCTTGCTAGCAGAAGC-3′; reverse, 5′-GCTTCTGCTAGCAAGGCCTTA GAAAA-3′; Hamilton et al. 1999), in a Ligation High DNA ligation kit (Toyobo) with the restriction enzyme *Xmn*I on a continuous cycle of 16°C for 30 min followed by 37°C for 10 min. After ligation, the polymerase chain reaction (PCR) was performed with the forward SNX primer under the following cycling profile: 94°C for 3 min; 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min; and a final 10 min at 72°C for last-strand elongation. The total volume of reaction buffer was 10 μL, which contained 0.5 μL of ligated DNA, 0.2 units of *Ex Taq*® polymerase (Takara), 0.2 mM dNTPs, and 0.4 μM primer. Amplified DNA fragments were hybridized with two 3′ biotinylated probes, $(AC)_{16}$ and $(TC)_{16}$, then were captured on streptavidin-coated magnetic beads (Promega) and eluted into TE buffer by denaturing at 95°C for 5 min.

The eluted DNA was again amplified with the forward SNX primer and subsequently cloned into the pGEM-T plasmid vector (Promega). After blue/white selection, the white colonies were checked for their length by means of PCR with the primers SP6 (5′-ATT-TAGGTGACACTATAGAATAC-3′) and T7 (5′-TAATACGACTCACTATAGGGCGA-3′) under the following cycling profile: 94°C for 3 min; 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min; and a final 10 min at 72°C for last-strand elongation. We sequenced 100 randomly chosen recombinant clones. If we detected microsatellite sequences in the inserts, we designed primers for the flanking regions using the Primer3 software (Rozen and Skaletsky 2000).

Estimation of population structure in a tea field

Study sites and mite sampling

We sampled the field population of *N. womersleyi* at four sites (A, B, C, and T) in an experimental tea field at the Mie Prefecture Agricultural Research Institute, Kameyama, Mie, Japan (34.872°N, 136.453°E), from August to October 2008 (Fig. 1). Sites A and B contained 24- to 25-year-old tea plants, and site C contained 6-year-old tea plants. Site T contained Mexican sunflowers, which were transplanted from a greenhouse in late April of the year. Each site was 10 to 20 m^2 in area. Chemical pesticides sprayed during our experiment were shown in Table 1, as well as dates of sampling phytoseiid mites. For sites A, B, and T, we collected the phytoseiid mites twice, and grouped them into subpopulations according to both the sampling site and the date (Table 1). For site C, we collected only three phytoseiid mites during an 18-day period, so we treated these as a single subpopulation in the analysis. Leaves infested with spider mites were taken into the laboratory, and we removed adult female *N. womersleyi* under binocular microscope and preserved them in vials containing 99.5% ethanol until DNA extraction.

Genotyping

Genomic DNA was individually extracted from the whole body of each adult female in a 0.5-mL microtube using the method described above. Genotyping PCR was carried out using the Type-it Microsatellite PCR Kit (Qiagen) in a total volume of 10 μL that contained 0.5 μL of mite DNA and 0.2 μM each primer. Two or three loci were simultaneously amplified in a single reaction. One of the primers for each locus was labeled with Beckman Dyes (Sigma–Aldrich). PCR was performed in an iCycler thermal cycler (Toyobo) under a cycling profile of 95°C for 5 min; 30 cycles of 95°C for 30 s, 60°C for 90 s, and 72°C for 30 s; and a final 30 min at 60°C for last-strand elongation. Fragment

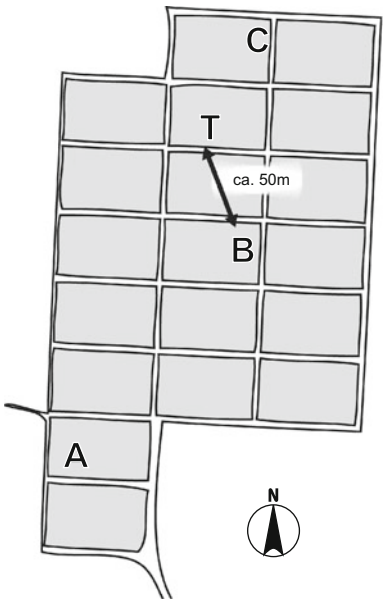

Fig. 1 Locations of the four sites in the tea field where we collected *Neoseiulus womersleyi*. Sampling dates were shown in Table 1

Table 1 Chemical pesticide sprayed in the tea field during our experiments

Date	Site A	B	C	T
22 May, 2008	Fenpyroximate, Buprofezin	Fenpyroximate, Buprofezin	Flubendiamide, Chlorfenapyr	
11 June	Acetamiprid	Acetamiprid		
18 June			Acetamiprid	
16 July			Imidacloprid, Iufenuron	
24 July	Cypermethrin	Cypermethrin		
24 July	Flubendiamide	Flubendiamide		
13 August		Sampling (B1)[a]		Sampling (T1)[a]
14 August	Sampling (A1)[a]			
15 August		Clothianidin, Pyridaben		Sampling (T2)[a]
16 August	Sampling (A2)[a]			
2 September		Sampling (B2)[a]		
12 September	Fenpyroximate, Emamectin benzoate		Permethrin, Acetamiprid	
17 September			Permethrin, Buprofezin	
18 September	Diafenthiuron	Diafenthiuron		
3 October			Sampling (C)[a]	
15 October			Sampling (C)[a]	
22 October			Sampling (C)[a]	

Dates of sampling *Neoseiulus womersleyi* were also described

[a] Sampling of *N. womersleyi*. Codes in parenthesis are used for each subpopulation in text

analysis was performed using a CEQ^{TM} 8000 Genetic Analysis System (Beckman Coulter) using 0.5 μL of the PCR products, 40 μL of deionized formamide, and 0.4 μL of 400-bp size standard (Beckman Coulter). The length of each amplified fragment was estimated using the software provided with the system, and fragments of different length were treated as different alleles.

Data analysis

Tests for Hardy–Weinberg equilibrium were performed using the Genepop version 4.0.10 software (Rousset 2008) with the default setting (10,000 dememorization steps, 20 batches, and 5,000 iterations per batch). Genetic diversity estimates, including expected (*He*) and observed (*Ho*) heterozygosities, were also calculated using Genepop. Allelic richness and linkage disequilibrium were computed using Fstat version 2.9.3 (Goudet 1995, 2001). Frequencies of null alleles were calculated using Genepop's expectation–maximization algorithm.

The fixation indexes (F_{ST}; Weir and Cockerham 1984) were calculated among subpopulations, which were grouped according to both the sampling site and the date, using Fstat version 2.9.3. We used a cluster analysis to investigate the genetic relationships

among subpopulations, computed by POPULATIONS version 1.2.32 (Langella 2002). The genetic distances were estimated by the Cavalli-Sforza and Edwards' (1967) chord distance (Dc). The resulting distance matrix was used to construct dendrogram with the neighbour-joining algorithm. The same analysis was performed on 1,000 bootstrapped datasets for both loci and individuals.

We then investigated patterns in the population genetic structure using the STRUCTURE version 2.3.1 software (Pritchard et al. 2000) and a Bayesian clustering approach at the individual level. The analysis was performed under the admixture model with correlated allele frequencies, following the method of Falush et al. (2003). We performed 20 independent runs for each K value (the number of suggested clusters), ranging from 1 to 30 clusters, with a burn-in period of 200,000 Markov-chain Monte Carlo (MCMC) repetitions followed by 200,000 MCMC repetitions for the actual analysis. We defined the number of clusters that best fit our data using both log posterior probabilities and ΔK values (Evanno et al. 2005). Once the most reliable K value was obtained, all individuals were assigned probabilistically to the K populations using 100 independent runs with a burn-in period of 200,000 MCMC repetitions followed by 200,000 MCMC repetitions for the actual analysis. In the final step, we averaged the results of the 100 runs using the Clumpp version 1.1.2 software (Jakobsson and Rosenberg 2007) and presented the results in the form of bar graphs using the Distruct version 1.1 software (Rosenberg 2004).

To analyze the population genetic structure, we estimated pairwise kinship coefficients (Loiselle et al. 1995) between individual mites using the SPAGeDi version 1.3a software (Hardy and Vekemans 2002), with the values compared between sampling sites and dates. For the spatial correlation analysis, we rounded off the distances between sampling sites to multiples of 50 m.

Results and discussion

Isolation of microsatellite loci

We found microsatellite repeat motifs in 89 of the 100 clones we sequenced. After eliminating identical clones and short repeats, we designed 14 pairs of primers. After screening the primer pairs using the laboratory strains of *N. womersleyi*, we found 10 loci that could be amplified consistently and used these loci for further analysis. Table 2 shows the primers, multiplex groups in which two or three loci were simultaneously amplified, repeat motifs found in the sequenced clones, and accession numbers deposited in the DDBJ/EMBL/GenBank databases. Our fragment analysis using 77 adult females of the tea field populations revealed that all 10 loci were polymorphic. Table 3 shows the characterization of each locus. The number of alleles per locus ranged from 10 at *NwMS828* to 58 at *NwMS801*. Allelic richness was also high, ranging from 2.902 to 3.871. Furthermore, we did not find linkage disequilibria between most pairs of loci (Table 4), so these markers can be treated as independent loci.

Understanding the dispersal and distribution patterns of phytoseiid mites is an essential tool for improving the conservation of indigenous phytoseiid mites to support their use in biological control. Although direct observation of such small organisms is difficult, molecular markers can be used to estimate their movement indirectly through the detection of gene flow (Slatkin 1987). However, for phytoseiid mites, effective genetic markers have not been established previously. Hinomoto and Maeda (2005) developed three microsatellite markers for *N. womersleyi*, and Maeda and Hinomoto (2006) analyzed the effect of

Table 2 Primer sequences, fluorescent dyes, groups simultaneously used for multiplex PCR, repeat motifs and accession numbers of the 10 microsatellite markers developed for *Neoseiulus womersleyi*

Locus	Forward primer[a]	Reverse primer	Group[b]	Repeat motif[c]	Accession no
NwMS801	D3-CCTACCGTTAACCTGGCGTA	GAAAGCGTGAGGAGTGGAAC	C	$(CT)_{16}$	AB533197
NwMS810	D2-GGATGAAGAGAGAGCGAGAAAGTAT	ACCTCCATTTTCTTCCTCCTT	A	$(AG)_{11}$	AB533198
NwMS814	D4-CGCGAGCGAGCTTGTTTT	GTCCTCTTCCGATCAACACC	D	$(CT)_{23}$	AB533199
NwMS828	D4-TTCATCTCTCGACCCTCTCC	GGAGGAAACTAGGAGCTGGA	B	$(TC)_{9}$	AB533200
NwMS831	D2-CAGAGAACGAGAAGAGATCAGG	CATCGTCAGACTTTGTTCCTGT	B	$(GA)_{8}$	AB533201
NwMS856	D3-CTGGAGCCCCTCGAAGTTTA	GGGCTCGAAAGGTTCAAAA	C	$(CT)_{12}$	AB533202
NwMS861	D4-TTCGTGAAATTCGTTGATCG	AGTGACGATTTCGCCTCAAA	C	$(TTTCTCTC)_{26}$	AB533203
NwMS867	D2-TTCGTCGTCTGTGGAAGTTG	AGCGCAATCGCTTCAAAGT	D	$(CT)_{10}$	AB533204
NwMS872	D4-ATGGCGATACGACGACAAA	CGCTCGCTGAACTCAAATAG	A	$(GA)_{24}$	AB533205
NwMS880	D2-CAAGTTTCCAGCTCGGTCAT	GCAGAAGGAGCTACTGAAGCA	D	$(CT)_{23}$	AB533206

[a] Beckman dyes were at the 5′ end

[b] Loci with same character are simultaneously amplified in the same PCR reaction

[c] From the sequenced clones

Table 3 Numbers of alleles observed (NA), observed allele size ranges (bp), allelic richness (AR), expected and observed heterozygosities (*He* and *Ho*, respectively), inbreeding coefficients (F_{IS}), and null allele frequencies (NF) for the 10 microsatellite markers obtained from the field population

Locus	NA	Size range (bp)	AR	*He*	*Ho*	F_{IS}	NF
NwMS801	58	108–452	3.871	75.307	63***	0.164	0.083
NwMS810	20	129–177	3.539	67.021	37***	0.450	0.226
NwMS814	11	58–86	3.254	42.134	13***	0.694	0.341
NwMS828	10	63–85	3.050	52.189	47	0.100	0.070
NwMS831	21	97–161	3.211	63.874	57	0.108	0.069
NwMS856	14	85–159	3.221	62.172	18***	0.712	0.332
NwMS861	11	137–203	2.902	49.128	17***	0.656	0.294
NwMS867	19	101–143	3.599	63.193	28***	0.559	0.272
NwMS872	13	61–89	3.400	65.973	64	0.030	0.017
NwMS880	22	118–166	3.374	66.114	52***	0.215	0.116

*** Significant difference between *He* and *Ho* ($P < 0.001$; Hardy–Weinberg exact test)

rearing conditions on the genetic diversity of laboratory strains by using these markers. Only three markers are probably too few to be used for field populations. The simple core repeats and uniform PCR temperature for the newly developed markers in the present study let us easily analyze polymorphisms.

Estimation of population structure in a tea field

The observed heterogeneity was lower than the expected heterogeneity at all loci, and the difference was significant for 7 of the 10 loci; the inbreeding coefficient (F_{IS}; Weir and Cockerham 1984) was also significant at several loci (Table 3). This may be mainly due to the presence of null alleles. However, as shown by locus *NwMS810*, F_{IS} was high despite a relatively low frequency of null alleles, suggesting the existence of the Wahlund effect. We could not determined the optimal number of clusters by STRUCTURE analysis because the mean value of the observed log-likelihood was high both at $K = 3$ and around $K = 10$ (Fig. 2a). Then, the approach of Evanno et al. (2005) indicated that three clusters was the most likely value ($K = 3$) because ΔK was remarkably higher at this number than at all other ΔK values (Fig. 2b). Thus, we conclude that the mite population was derived from three genetic clusters. Figure 3 shows the results of the clustering analysis and individual assignment analysis for $K = 3$. Most of the individuals collected at site A were assigned to the same cluster (shown in white in the bar graphs in Fig. 3). Individuals collected at other sites could not be clearly assigned into a single cluster, although the probabilities of being assigned to the "white" cluster were generally low (Fig. 3). These results implied that the subpopulation A1 and A2 were genetically distinct from other subpopulations.

To detect subpopulation differentiation statistically, the fixation indexes F_{ST} among subpopulation were calculated (Table 5). No significant differentiation among subpopulations was found, suggesting the differentiation among subpopulations was very low. However, neighbour-joining tree constracted based on Cavalli-Sforza and Edwards' distance supported that genetic similarity between subpopulations A1 and A2 (Fig. 4), showing mites collected on site A were remarkably characteristics. Subpopulations B1 and T1, and C1 and T2, were also similar, suggesting that gene flow among these three sites occurred.

Table 4 Results of tests for genotypic disequilibrium between pairs of loci developed for *Neoseiulus womersleyi*

	NwMS810	*NwMS814*	*NwMS828*	*NwMS831*	*NwMS856*	*NwMS861*	*NwMS867*	*NwMS872*	*NwMS880*
NwMS801	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0567	1.0000	1.0000
NwMS810		1.0000	1.0000	0.0989	0.2378	0.8489	0.1400	1.0000	0.0800
NwMS814			0.5844	1.0000	0.6222	1.0000	0.2356	1.0000	0.4733
NwMS828				0.4989	0.8233	0.7522	1.0000	0.1622	1.0000
NwMS831					0.1744	0.4833	1.0000	0.4200	0.2167
NwMS856						0.3378	0.0389*	0.3156	0.0222*
NwMS861							0.1533	0.2622	0.8567
NwMS867								1.0000	1.0000
NwMS872									1.0000

The probable independence of each pair of loci is shown

* Significant genotypic disequilibrium ($P < 0.05$)

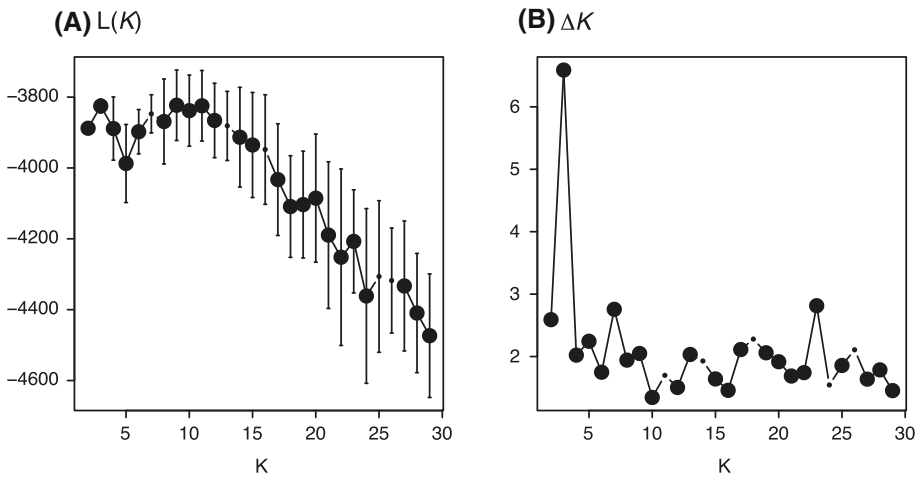

Fig. 2 Graphical inference to estimate the number of genetic clusters using the STRUCTURE software. (**A**) Mean log-likelihood values [L(*K*)] ± SD as a function of *K*, for $K = 1$ to $K = 30$, where *K* represents the number of clusters. (**B**) Rate of change in the log-likelihood of the data (ΔK; Evanno et al. 2005) as a function of *K*

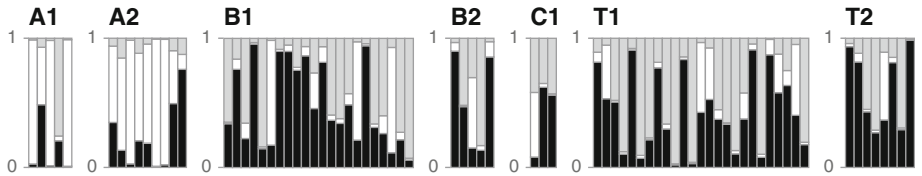

Fig. 3 The results of Bayesian clustering analysis and individual assignment analysis of *Neoseiulus womersleyi* using the STRUCTURE software for three clusters. The *x*-axis of the bar chars represents individual mites. The *y*-axis of the bar chars represents the individual assignment probabilities. *Black*, *grey*, and *white* components of each bar represent the proportion in each of the three clusters

Table 5 Multilocus estimates of pairwise F_{ST} (above diagonal) and pairwise significance after standard Bonferroni corrections by overall loci *G*-statistics (below diagonal) among subpopulations of *Neoseiulus womersleyi*

	T1	T2	B1	B2	A1	A2	C1
T1		0.0082	0.0143	−0.0037	0.0346	0.0386	0.0399
T2	NS		0.0161	0.0172	0.0144	0.0432	0.0182
B1	NS	NS		−0.0155	0.0187	0.0442	0.0636
B2	NS	NS	NS		0.0058	0.0261	0.0482
A1	NS	NS	NS	NS		−0.0075	0.0417
A2	NS	NS	NS	NS	NS		0.0935
C1	NS	NS	NS	NS	NS	NS	

G-statistics were calculated based on allele frequencies after correction of null alleles. Significance levels were determined by 420 permutations. *NS* not significant

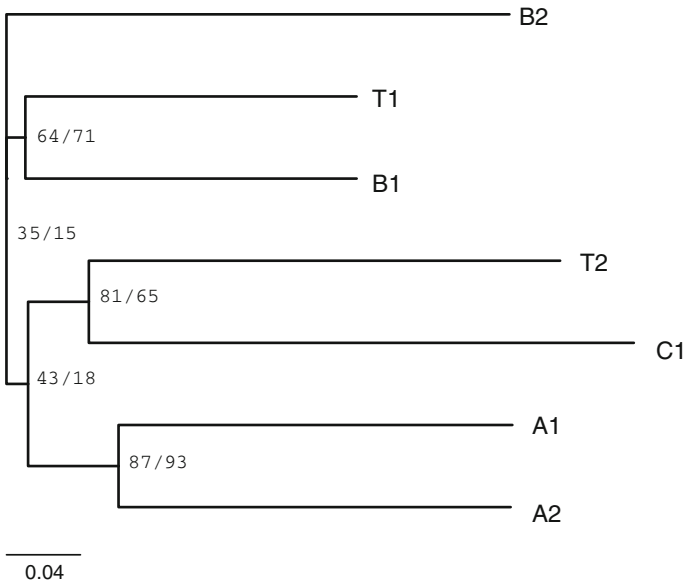

Fig. 4 Neighbour–joining tree for seven subpopulations of *Neoseiulus womersleyi* collected in the tea field based on the Cavalli-Sforza and Edwards' (1967) chord distance (Dc). Numbers are bootstrap support indices on loci (*left*) and on individuals (*right*), respectively

Although the clear difference between subpopulations were not detected, at the individual level, we found a negative correlation between kinship coefficients and the geographic distance ($P < 0.001$; Kendall's rank correlation, $\tau = -0.0450$) (Fig. 5). The coefficients differed significantly among distance ($P < 0.001$; Kruskal–Wallis rank-sum test), implying that *N. womersleyi* gradually disperse in this tea field, possibly by walking as shown in Todokoro and Isobe (2010).

In the present study, several pesticides had been sprayed in the experimental field during our research (Table 1). For example, pyridaben (SANMITE SC, Nissan Chemical Industories), which is known to severely reduce the survival of phytoseiid mites (Amano et al. 2004), was used at site B between our first and second samplings. Nevertheless, subpopulation B2 did not genetically differ from subpopulation B1, and collections from site T (T1 and T2) were also similar to B2 (Fig. 3). There are two possible explanations for this: (1) the effect of pyridaben on the phytoseiid mites was incomplete and the B2 mites were survivors of the spray, and (2) the mites at site B were completely eradicated by the pyridaben, thus the B2 mites came from other sites, possibly site T, where no pesticides were sprayed. Todokoro and Isobe (2010) estimated from the population dynamics of *N. womersleyi* that their dispersal rate was ca. 4 m per 10 days in tea fields. This result suggested that *N. womersleyi* mainly dispersed by walking in tea fields. Because sites B and T were about 50 m apart (Fig. 1) and the time elapsed after the spraying was only 18 days, it is not likely that the B2 mites came from site T. From these results, the B2 mites appear to be survivors of the spraying or their offspring.

The assignment patterns of individuals were most similar between sites B and T and between sites C and T (ca. 50 m apart) and between sites B and C (ca. 100 m apart) (Fig. 1), suggesting that *N. womersleyi* seems to disperse within a radius of around 100 m. Although it is known that phytoseiid mites can travel long distances by means of aerial

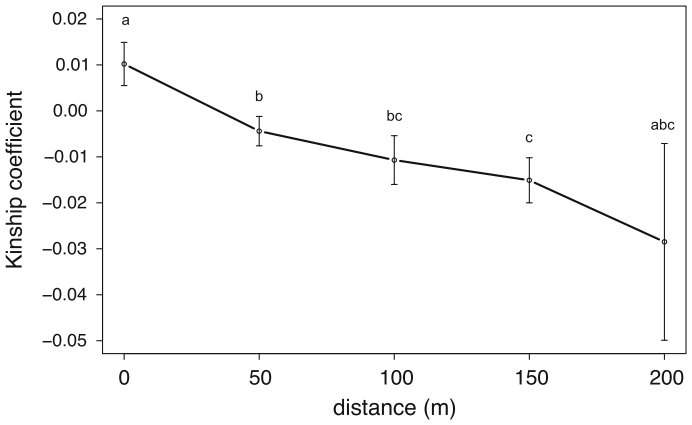

Fig. 5 The average and SD of the kinship coefficients (Loiselle et al. 1995) between individual *Neoseiulus womersleyi* grouped into each 50-m distance. Points labelled with different letters differ significantly ($P < 0.05$; pairwise comparisons using the Wilcoxon rank-sum test adjusted using Holm's method)

dispersal (Hoy et al. 1985; Croft and Jung 2001; Tixier et al. 1998), the assignment pattern for the mites collected at site A, which was more than 100 m from the other sites, appeared to be distinct from the patterns at the other sites, indicating that the mites rarely disperse farther than 100 m in tea fields such as those at the study sites. On the other hand, the assignment pattern for the mites collected at site T, where Mexican sunflowers had been experimentally planted to help preserve *N. womersleyi*, were similar to those at sites B and C, supporting the hypothesis that the mite populations would increase on the Mexican sunflowers and disperse to the adjoining tea plants. Our study therefore suggests that the Mexican sunflowers and the tea plants should be planted in each 100-m units to conserve the indigenous phytoseiid mites and help them to disperse to the tea plants in this field. In order to make this technique more reliable, however, further case studies will be required.

Fine-scale analysis of the population structure using microsatellite markers has been conducted in some species of spider mites (Navajas et al. 2002; Nishimura et al. 2005; Uesugi et al. 2009a, b). Uesugi et al. (2009b) demonstrated frequent gene flow within field populations of spider mites. Our study suggested that populations of phytoseiid mites were stable in the evergreen tea fields. In this case, artificial manipulation of natural enemies can function effectively as a "push–pull strategy" (Cook et al. 2007). Attraction of natural enemies in the filed using synthetic herbivore-induced plant volatiles (HIPV) has also been attempted, and is expected to enhance biological control efforts (James 2003, 2005; Yu et al. 2008; Khan et al. 2008). Recently Ishiwari et al. (2007) identified three components of HIPV induced in tea plants infested with *T. kanzawai*, and all three were essential to attract *N. womersleyi*. If these chemicals are placed in tea fields, *N. womersleyi* is likely to colonize the fields and help to control *T. kanzawai*. Although the effective distance over which the volatiles can attract the mite is not yet clear, chemical attraction of *N. womersleyi* will enhance the biological control of *T. kanzawai* if a clear understanding of the population structure of *N. womersleyi* can guide the deployment of these attractants. The information and techniques for estimating mite dispersal that were demonstrated in the present study will also help to plan habitat management for the conservation of natural enemies.

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