



Dominant honeybee colony infestation by *Varroa destructor* (Acari: Varroidae) K haplotype in Japan

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Abstract

Varroa destructor Anderson and Trueman (Acari: Varroidae) are ectoparasitic mites found in the western honeybee *Apis mellifera* Linnaeus (Hymenoptera: Apidae). *Varroa destructor* is classified into two haplotypes, i.e., Korea (K) and Japan (J), based on mtDNA sequences. Among these, *V. destructor* K haplotype is possibly a more severe threat to *A. mellifera* colonies. Previous studies collected both *V. destructor* haplotypes from honeybee colonies in Japan. However, no detailed surveillance of infestation of Japanese apiaries by *V. destructor* or identification of their genetic structure has been conducted to date. We surveyed *V. destructor* at 15 different Japanese apiaries of *A. mellifera*. *Varroa destructor* was collected from 14 Japanese apiaries, and all mites were classified as *V. destructor* K haplotype. *Varroa destructor* infestation of the Japanese honeybee *A. cerana japonica* Radoszkawski (Hymenoptera: Apidae) was also analyzed. *Varroa destructor* K haplotype was predominant in *A. cerana* colonies. Despite the different host species, all collected *V. destructor* K haplotype samples were classified into a single haplogroup, i.e., K1-1/K1-2. These results indicate that *A. mellifera* and *A. cerana* were infested by the same *V. destructor* haplogroup. This is the first report detailing a survey on *V. destructor* prevalence and haplogroups among Japanese apiaries.

Keywords *Varroa destructor* · *Apis mellifera* · *Apis cerana* · Haplotype · Beekeeping

Introduction

Varroa destructor Anderson and Trueman (Acari: Varroidae) are known as ectoparasitic mites of the western honeybee *Apis mellifera* Linnaeus (Hymenoptera: Apidae). *Varroa destructor* proliferates in beehives, and it spends its phoretic phase on adult honeybees and reproductive phase on pupal honeybees (Dietemann et al. 2015; Evans and Cook 2018). Although *V. destructor* feeds on the hemolymph of immature and adult honeybees, a recent study demonstrated that *V. destructor* primarily feeds on the adipose tissue of honeybees (Ramsey et al. 2019). *Varroa destructor* transmits several viruses that cause severe diseases in honeybees such as deformed wing virus (Dainat et al. 2012; Hedtke et al. 2011; Martin 2001; Nazzi et al. 2012). Physical damage and

disease transmission by *V. destructor* can cause fatalities not only of individual bees but also of entire honeybee colonies (Dainat et al. 2012; De Jong et al. 1982; Duay et al. 2002, 2003; Garedeew et al. 2004; Guzmán-Novoa et al. 2010; Nazzi et al. 2012; Ratti et al. 2015; Schneider and Drescher 1987). Consequently, *V. destructor* infestation has become a major cause of colony loss, particularly winter colony loss (Branco et al. 1999; Shimanuki et al. 1994). As in other countries, *V. destructor* infestation caused varroosis in Japan (MAFF 2018) and may be responsible for winter colony loss. However, detailed surveillance of *V. destructor* infestation, such as its seasonal prevalence and infestation level in each colony, has not been conducted in Japan.

Varroa destructor was initially mistaken for *V. jacobsoni* Oudemans (Acari: Varroidae), which is the primary parasite of the eastern honeybee *A. cerana* Radoszkawski (Hymenoptera: Apidae). Anderson and Truman (2000) clarified the morphological and genetic differences between *V. destructor* infesting *A. mellifera* and *A. cerana* colonies, and they termed the species that infests *A. mellifera* as *V. destructor*. *Varroa destructor* originally infested *A. cerana* colonies, but it has rarely caused severe damage to the host (Boot et al.

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1997; Fries et al. 1996). It has since expanded its host range to *A. mellifera* colonies and has become a severe pest of the novel host. Japan is believed to be one of the locations where *V. destructor* has spread to *A. mellifera* colonies through the introduction of Western beekeeping (Sakai and Okada 1973; Yoshiyama and Kimura 2018). Another region is the far east of the former USSR (Crane 1978).

Genetic analysis demonstrated that these mites have different mtDNA sequences (Solignac et al. 2005), thereby being termed as J (Japan) or K (Korea or Russia in earlier studies) haplotypes according to the collection region. J and K haplotypes vary in terms of mtDNA cytochrome oxidase I (*cox I*) (Anderson and Truman 2000; Solignac et al. 2005). These differences have been investigated using randomly amplified polymorphic DNA or restriction fragment length polymorphism (RFLP)/polymerase chain reaction (PCR) of mtDNA (Anderson and Fuchs 1998; Kraus and Hunt 1995). *Varroa destructor* K haplotype is suspected to have a higher virulence and reproductive ability than its J haplotype (Delfinado-Baker 1988; Garrido et al. 2003; Strapazzon et al. 2009).

Varroa destructor J and K haplotypes were further classified into several haplogroups according to variations in *cox I* and *III*, *ATP synthase 6 (atp 6)*, and *cytochrome b (cyt b)* sequences in mtDNA (Navajas et al. 2010) discovered following whole *V. destructor* mtDNA sequencing (Evans and Lopez 2002; Navajas et al. 2002). *Varroa destructor* J haplotype includes six haplogroups (J1-1–J1-6), whereas its K haplotype includes four haplogroups (K1-1–K1-4). Although Navajas et al. (2010) distinguished between K1-1 and K1-2, no differences were observed in terms of the registered sequences of *cox I*, *cox III*, *atp 6*, and *cyt b* between them. Therefore, they are described as K1-1/K1-2 in this study. Phylogenetic analysis of mtDNA sequences between these haplogroups showed that *V. destructor* K and J haplotypes formed distinct clades, supporting the fact that *V. destructor* K or J haplotype independently expanded their host range from *A. cerana* to *A. mellifera* (Navajas et al. 2010).

Varroa destructor is now globally dispersed, and its K haplotype represents the primary haplogroups worldwide. Studies conducted in the early twenty-first century reported that *V. destructor* K haplotype was widely detected in Asia, Europe, South and North America, and Oceania (New Zealand). *Varroa destructor* K haplotype infestations are still reported in several countries including Iran, Serbia, Benin, and Turkey (Farjamfar et al. 2018; Gajic et al. 2013, 2016; Kelomey et al. 2017; Warrit et al. 2015). Although *V. destructor* J haplotype has been found in a limited number of countries including Japan, Thailand, several countries in North and South America, and Spain (Anderson and Trueman 2000; de Guzman et al. 1997, 1999; Guerra et al. 2010; Maggi et al. 2012; Muñoz et al. 2008; Navajas et al. 2010;

Solignac et al. 2005; Warrit et al. 2006), they have rarely been reported recently. However, the existence of *V. destructor* K haplotype has been reported in Brazil, which was first invaded by *V. destructor* J haplotype (Garrido et al. 2003; Strapazzon et al. 2009). In addition to *V. destructor* K and J haplotypes, regional *V. destructor* haplotypes have also been observed (Gajic et al. 2013, 2016; Navajas et al. 2010; Zhou et al. 2004), although their spread to other countries has not been reported.

In Japan, both these haplotypes have been collected from *A. mellifera* and *A. cerana* colonies (Navajas et al. 2010; Solignac et al. 2005). These reports determined that these haplotypes have genetic differences and are regionally distributed on a global scale. Navajas et al. (2010) analyzed three *V. destructor* samples collected from two *A. mellifera* and three *A. cerana* colonies in Japan during 1994–2000. The report showed that *V. destructor* samples collected from *A. mellifera* colonies were J or K haplotype (J1-6 in Tokyo and K1-1 in Tokyo), whereas those collected from *A. cerana* colonies were J haplotypes (J1-2 in Tokyo, J1-3 in Machida, and J1-4 in Shikoku) (Fig. 1a). Solignac et al. (2005) analyzed 1–19 *V. destructor* samples collected from two *A. cerana* and five *A. mellifera* colonies in Japan. Several colonies had single haplotypes: three *A. mellifera* colonies had *V. destructor* K haplotype (Yatsushiro, Yokohama, and Tokyo) and a single *A. cerana* colony had *V. destructor* J haplotype (Yatsushiro). The three remaining colonies had coexisting *V. destructor* K and J haplotypes in the same honeybee population (*A. cerana* colony in Machida and *A. mellifera* colonies in Machida and Noda). There were more K haplogroups than J haplogroups in these three colonies (Solignac et al. 2005). In addition, Techer et al. (2019) collected K1-1/K1-2 haplogroups from *A. mellifera* colonies in Okinawa Prefecture. Although the distribution of K haplogroups is irregular, no surveillance of the actual genetic structure of *V. destructor* in Japan has been conducted. To understand the significance of *V. destructor* infestation of *A. mellifera* colonies in Japan, apiaries across Japan were surveyed for *V. destructor* and the haplogroups of the collected *V. destructor* samples were determined. To the best of our knowledge, this is the first survey on the detailed genetic structure of *V. destructor* populations among Japanese apiaries.

Materials and methods

Collection of *V. destructor* from honeybee colonies

Varroa destructor females were collected from July to October 2018 from 15 different commercial apiaries located in Hokkaido, Fukushima, and Osaka Prefectures in Japan (Table 1, Fig. 1b). Phoretic *V. destructor* samples were collected using a modified sugar roll test (Ogihara

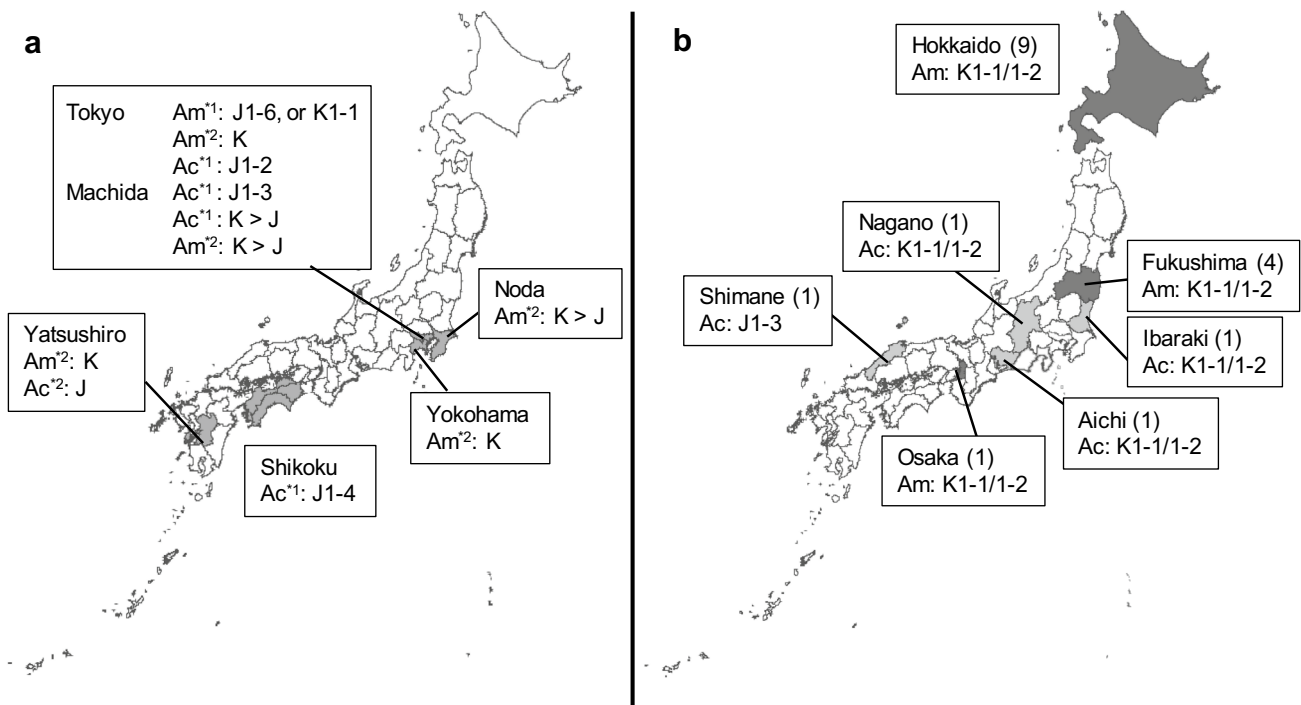


Fig. 1 Distribution of *Varroa destructor* haplotypes and haplogroups in Japan. **a** Summary of *V. destructor* haplotypes and haplogroups reported by Navajas et al. (2010) and Solignac et al. (2005). *1: data from the study by Navajas et al. (2010). *2: data from the study by Solignac et al. (2005). **b** *Varroa destructor* haplogroups determined

in the current study. The numbers in parentheses represent the numbers of apiaries infested with *V. destructor*. Black areas indicate the surveillance locations for *Apis mellifera* (Am) colonies, and gray areas indicate surveillance locations for *A. cerana* (Ac) colonies

Table 1 Sites and dates of *Varroa destructor* mite collection from *Apis mellifera*

Apiary	Site of collection	Principal location	Date of sample collection	Migratory/stationary
Hokkaido 1	Hokkaido	Hokkaido	Jul 18, 2018	Stationary
Hokkaido 2	Hokkaido	Hokkaido	Jul 18, 2018	Migratory
Hokkaido 3	Hokkaido	Hokkaido	Jul 19, 2018	Migratory
Hokkaido 4	Hokkaido	Aichi	Jul 17, 2018	Migratory
Hokkaido 5	Hokkaido	Wakayama	Jul 18, 2018	Migratory
Hokkaido 6	Hokkaido	Wakayama	Jul 17, 2018	Migratory
Hokkaido 7	Hokkaido	Hokkaido	Aug 8, 2018	Migratory
Hokkaido 8	Hokkaido	Wakayama	Aug 8, 2018	Migratory
Hokkaido 9	Hokkaido	Mie	Aug 8, 2018	Migratory
Hokkaido 10	Hokkaido	Kagoshima	Aug 6, 2018	Migratory
Fukushima 1	Fukushima	Fukushima	Sept 12, 2018	Migratory
Fukushima 2	Fukushima	Fukushima	Sept 12, 2018	Migratory
Fukushima 3	Fukushima	Fukushima	Sept 12, 2018	Migratory
Fukushima 4	Fukushima	Fukushima	Sept 12, 2018	Migratory
Osaka 1	Osaka	Osaka	Oct 24, 2018	Migratory

et al. submitted). In brief, 1 cup (200 ml, approximately 500 honeybees) of adult honeybees was collected from individual beehives. Honeybees were put into a plastic jar (240 mm long, 65 mm I.D.) containing 100 g of powdered sugar (Uehara, Tokyo, Japan) and shaken for 1 min. Dislodged *V. destructor* and powdered sugar were sifted

through a rough mesh (3 mm) attached to a hole punched on the jar lid. *Varroa destructor* samples were collected using a fine screen and counted. Then 9–12 beehives were surveyed at each apiary. For genetic analysis, *V. destructor* samples were collected from capped cells in a honeycomb

frame when small numbers of *V. destructor* samples were obtained using the sugar roll test.

Varroa destructor-infested *A. cerana* colonies were kindly gifted by Dr. Taro Maeda (Institute of Agrobiological Sciences, National Agriculture and Food Research Organization). *A. cerana* beehives are located at Tsukuba and Kashima (Ibaraki Prefecture), Inuyama (Aichi Prefecture), Matsumoto (Nagano Prefecture), and Matsue (Shimane Prefecture; Fig. 1b). Only a single *V. destructor* was obtained from each location. The Japanese map shown in Fig. 1 was constructed using Excel for Office 365 MSO (Microsoft, Redmond, WA, USA).

Analysis of *V. destructor* haplotypes and haplogroups by RFLP/PCR and sequencing

Genomic DNA was extracted from individual *V. destructor* using the Easy DNA Extraction Kit version 2 (Kaneka Corporation, Tokyo, Japan). *Varroa destructor* haplotypes were determined using RFLP/PCR, which was a slightly modified form of the method reported by Solignac et al. (2005). The obtained genomic DNA was amplified with GoTaq Green Master Mix (Promega, Fitchburg, USA) using COIF-long and COIR primers (Table 2). PCR conditions were as follows: denaturing at 95 °C for 2 min, 35 cycles of denaturing at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s. PCR products were digested with the restriction enzyme *Sac* I (Takara Bio Inc., Shiga, Japan) at 37 °C. Digestion was confirmed using electrophoresis.

To determine the *V. destructor* haplogroups in Japan, *cox I*, *cox III*, *atp 6*, and *cyt b* mtDNA sequences were determined using the primers described by Navajas et al. (2010). PCR was performed using KOD FX Neo (TOYOBO, Osaka, Japan). PCR conditions were as follows: denaturing at 94 °C for 2 min, 35 cycles of denaturing at 98 °C for 10 s, annealing at 50 °C for 30 s, and extension at 68 °C for 45 s. Obtained PCR products were purified using the QIAquick PCR Purification Kit, QIAGEN II Gel Extraction Kit (QIAGEN, Hilden, Germany), or Ethachinmate (Nippon gene,

Tokyo, Japan). Then direct sequencing was performed using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, USA) on an ABI 3500xl Genetic Analyzer (Applied Biosystems, Waltham, USA).

Statistical analysis

Spearman's rank correlation analysis was performed using R software (version 3.5.1; R Developmental Core Team 2013). Correlation analysis between the average number of dislodged *V. destructor* among the infested colonies at each apiary and the ratio of infested honeybee colonies at each apiary were analyzed using Spearman's rank correlation method.

Results

Surveillance of *V. destructor* infestation at Japanese apiaries

Varroa destructor samples were collected from *A. mellifera* colonies located at 15 different commercial apiaries using modified phoretic mite detection. In Japan, many beekeepers migrate to Hokkaido Prefecture in summer for honey collection and colony rearing. Therefore, surveillance was primarily conducted in Hokkaido Prefecture (10 apiaries). Four apiaries in Fukushima Prefecture and one apiary in Osaka Prefecture were also surveyed. These apiaries were not shifted to Hokkaido Prefecture (Table 1). *Varroa destructor* samples were collected from 14 apiaries (Fig. 2), except for the Hokkaido 7 apiary. The number of *V. destructor* dislodged by each sugar roll test was greater in the later season (Fig. 2). Similar to the number of dislodged *V. destructor*, the ratio of *V. destructor*-infested honeybee colonies at each apiary was higher in autumn than in summer (Fig. 2). The average number of dislodged *V. destructor* among the infested honeybee colonies correlated with the ratio of *V. destructor*-infested honeybee colonies at the apiaries

Table 2 Primers used in this study

Experiment	Sequence (5' → 3')	References
RFLP/PCR		
CoIF	TACAAAGAGGGAAGAAGCAGCC	Solignac et al. (2005)
CoIR	GCCCCTATTCTTAATACATAGTGAAAATG	Solignac et al. (2005)
mtDNA sequencing		
CoxIF-long	CTTGTAATCATAAGGATATTGGAAC	Navajas et al. (2010)
CoIR	GCCCCTATTCTTAATACATAGTGAAAATG	Navajas et al. (2010)
atp6-cox3F	GACATATATCAGTAACAATGAG	Navajas et al. (2010)
atp6-cox3R	GACTCCAAGTAATAGTAAAACC	Navajas et al. (2010)
cytbF	GCAGCTTTAGTGGATTTACCTAC	Navajas et al. (2010)
cytbR	CTACAGGACACGATCCCAAG	Navajas et al. (2010)

Fig. 2 *Varroa destructor* surveillance at Japanese apiaries. The number of *V. destructor* dislodged in single sugar roll test in each colony is represented by open circles. *nd* not detected

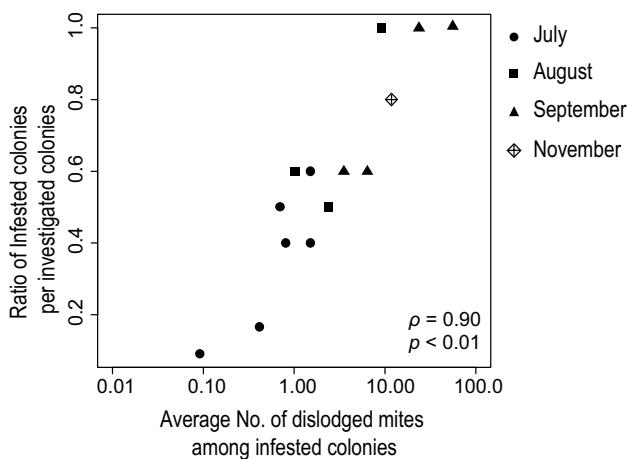
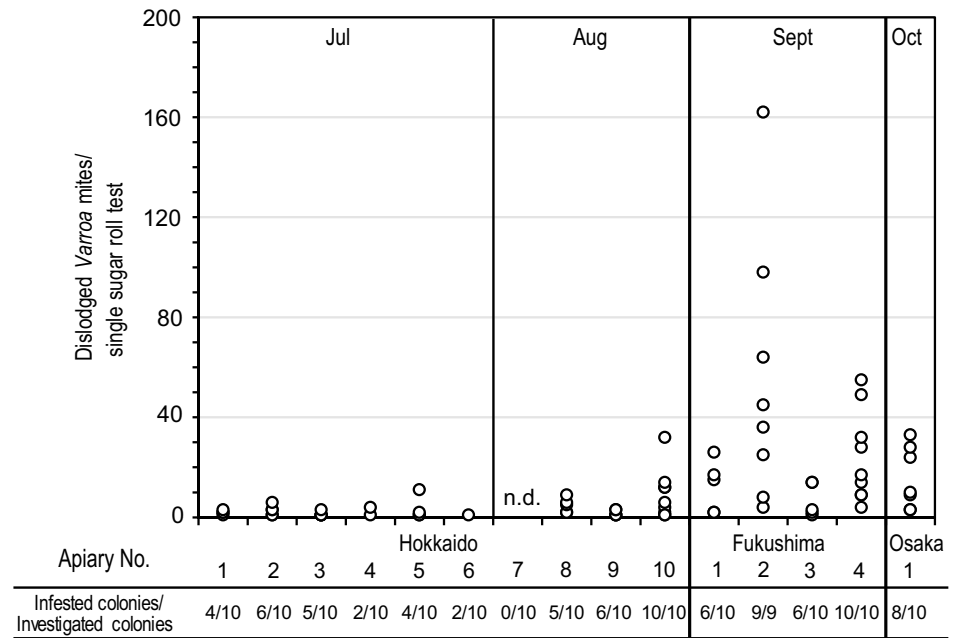


Fig. 3 Correlation of the average number of dislodged *Varroa destructor* among infested honeybee colonies at each apiary and the ratio of infested honeybee colonies at each apiary

($\rho = 0.90$, $p < 0.01$; Fig. 3), suggesting that *V. destructor* populations increase in summer and autumn.

Several reports have indicated that the threshold for the number of *V. destructor* for winter colony loss is 4–8 phoretic *V. destructor* per 100 honeybees in autumn (Currie and Gatien 2006; Gatien and Currie 2003; Strange and Sheppard 2001). Therefore, we set a strict benchmark of 20 dislodged *V. destructor* per single sugar roll test for predicting winter colony loss. In July and August, <20 *V. destructor* individuals were dislodged in a single sugar roll test, except for the Hokkaido 10 apiary. In contrast, cases with >20 *V. destructor* per sugar roll test increased at the apiaries surveyed

during September and October. In a severe case, 162 *V. destructor* individuals were detected in a single sugar roll test at the Fukushima 2 apiary. These colonies had a high probability of winter colony loss.

Genetic analysis of *V. destructor* haplotypes and haplogroups in Japan

Varroa destructor haplotypes were investigated using RFLP/PCR for mtDNA *cox I* (Solignac et al. 2005). All *V. destructor* samples collected in this study were of *V. destructor* K haplotype (Table 3). K1-1/K1-2, K1-3, and K1-4 showed variation in terms of *cox I* mtDNA sequence (Navajas et al. 2010). Therefore, the *V. destructor* haplogroups collected were investigated using mtDNA sequencing. All *V. destructor* samples collected in this study had mtDNA sequences identical to those of K1-1/K1-2 (Table 3). Partial sequences of *atp 6* to *cox III*, and *cyt b* were also determined to distinguish other regional *V. destructor* haplogroups (Navajas et al. 2010). However, no variations were observed for these mtDNA sequences. These data demonstrate that *V. destructor* infesting *A. mellifera* at Japanese apiaries were of *V. destructor* K1-1/K1-2 and were identical among all apiaries (Fig. 1b).

Varroa destructor infesting *A. cerana* was also obtained, and the haplotypes were analyzed using the same method as used for *A. mellifera* colonies. *Varroa destructor* samples collected from *A. cerana* colonies in Tsukuba, Kashima, Inuyama, and Matsue were *V. destructor* K1-1/K1-2 (Table 3, Fig. 1b). No sequence variations within *atp 6* to *cox III* or *cyt b* were found in these mites. This indicates

Table 3 *Varroa destructor* haplogroups in Japan

Species	Apiary	No. of investigated <i>V. destructor</i>	Haplotype			
			No. of K	K	No. of J	J
<i>Apis mellifera</i>	Hokkaido 1	10	10	K1-1/K1-2	–	–
	Hokkaido 2	10	10	K1-1/K1-2	–	–
	Hokkaido 3	10	10	K1-1/K1-2	–	–
	Hokkaido 4	10	10	K1-1/K1-2	–	–
	Hokkaido 5	10	10	K1-1/K1-2	–	–
	Hokkaido 6	10	10	K1-1/K1-2	–	–
	Hokkaido 7	No <i>V. destructor</i> was detected*				
	Hokkaido 8	10	10	K1-1/K1-2	–	–
	Hokkaido 9	10	10	K1-1/K1-2	–	–
	Hokkaido 10	10	10	K1-1/K1-2	–	–
	Fukushima 1	10	10	K1-1/K1-2	–	–
	Fukushima 2	10	10	K1-1/K1-2	–	–
	Fukushima 3	10	10	K1-1/K1-2	–	–
	Fukushima 4	10	10	K1-1/K1-2	–	–
Osaka 1	9	9	K1-1/K1-2	–	–	
<i>A. cerana</i>	Tsukuba	1	1	K1-1/K1-2	–	–
	Kashima	1	1	K1-1/K1-2	–	–
	Matsumoto	1	1	K1-1/K1-2	–	–
	Inuyama	1	1	K1-1/K1-2	–	–
	Matsue	1	–	–	1	J1-3

*No phoretic *V. destructor* was detected by sugar roll tests

that *A. mellifera* and *A. cerana* colonies were infested with the same *V. destructor* K1-1/K1-2 haplogroup. Only one *V. destructor* collected from Matsue (Shimane Prefecture) was *V. destructor* J haplotype. The mtDNA sequences of *cox I*, *atp 6* to *cox III*, and *cyt b* indicated that the haplogroup of *V. destructor* collected from Matsue was J1-3 with no differences compared to the original J1-3 (Table 3). Although we used a small number of samples, these results indicate that K1-1/K1-2 is the dominant *V. destructor* haplogroup causing damage to *A. cerana* in Japan (Fig. 1b).

Discussion

In this study, we conducted a survey on *V. destructor* infestation at 15 different Japanese apiaries. Although *V. destructor* has previously been collected from *A. mellifera* and *A. cerana* colonies (Navajas et al. 2010; Solignac et al. 2005; Techer et al. 2019), a field survey on *V. destructor* among Japanese apiaries has not been conducted previously. We could collect *V. destructor* from almost all investigated apiaries. The number of detected *V. destructor* in the colonies and the ratio of infested colonies at the apiaries were correlated and greater in autumn surveillance than that in summer (Fig. 3), indicating that *V. destructor* prevalence increased

in autumn. The number of *V. destructor* increased in summer to autumn in Japan, which has also been reported in other countries (Currie and Gatién 2006; Gatién and Currie 2003; Martin 2001). Some honeybee colonies had severe *V. destructor* infestations with more than 20 *V. destructor* detected using the sugar roll test. These colonies had *V. destructor* infestation that exceeded the benchmark for *V. destructor* number in terms of winter colony loss (Currie and Gatién 2006; Gatién and Currie 2003; Strange and Sheppard 2001); therefore, they had the potential to undergo winter colony loss by *V. destructor* infestation.

Varroa destructor infestation is compounded at an apiary in several manners such as drifting and robbing (Boecking and Genersch 2008). *Varroa destructor* infestation has been shown to disrupt the orientation of foragers and increase the frequency of drifters (Kralj and Fuchs 2006; Kralj et al. 2007), with highly infested colonies beginning to accept drifters (Forfert et al. 2015). *Varroa destructor* can serve as a vector for several viruses causing severe diseases such as deformed wing virus and acute paralysis virus (Boecking and Genersch 2008). Colonies with severe mite infestations appear to be the source of *V. destructor* and related disease at apiaries. Early treatment before an increase in *V. destructor* population in honeybee colonies is necessary to prevent the high probability of winter colony loss (van Dooremalen

et al. 2012). In Japan, *V. destructor* population is controlled with the acaricides τ -fluvalinate and amitraz. However, these acaricides cannot be used during honey production. It is common for beekeepers in Japan to continue honey production during September, which may allow an increase in *V. destructor* population that exceeds the threshold for winter colony loss. Monitoring *V. destructor* population and early acaricide-based treatment before an increase in its population may prevent severe *V. destructor* infestation.

The *V. destructor* haplotypes collected in this study were analyzed. *Varroa destructor* K haplotype was dominant in Japanese honeybee colonies. *Varroa destructor* K1-1 and J1-6 have previously been collected from *A. mellifera* colonies in Japan (Navajas et al. 2010). However, we demonstrated that *V. destructor* K1-1/K1-2 could be detected in *A. mellifera* colonies across all sampling locations. Although several reports have shown regional variations in mtDNA sequences (Gajic et al. 2013; Navajas et al. 2010; Zhou et al. 2004), no regional variations were found in the current study. In addition, Techer et al. (2019) reported that *V. destructor* in Okinawa Prefecture was *V. destructor* K1-1/K1-2, indicating this haplogroup is dominant in *A. mellifera* colonies in Japan. In contrast, *V. destructor* J haplotype appears to be decreasing at Japanese apiaries. Replacement of J haplotype with K haplotype has been reported in Brazil, concurrent with an increase in the fertilization rate of *V. destructor* observed in *A. mellifera* colonies (Garrido et al. 2003; Strapazzon et al. 2009). This suggests that *V. destructor* K haplotype has more severe impacts on *A. mellifera* colonies. The current results indicate that *V. destructor* K1-1/K1-2 has a higher infectivity than *V. destructor* J haplotype and can cause a loss of *V. destructor* diversity.

In Japan, *A. mellifera* colonies travel across various locations. Some *A. mellifera* colonies travel for the promotion of pollination for crop production such as strawberry horticulture, whereas some travel for migratory beekeeping practices (Yoshiyama and Kimura 2018). Hokkaido Prefecture is the major area for migratory beekeeping in Japan. Seventeen percent of all western honeybee colonies in Japan migrate to Hokkaido Prefecture from summer to autumn for honey production and for colony rearing (Livestock Farming Promotion Division, Bureau for Promotion of Agricultural Production of Hokkaido Pref 2018). Indeed, beekeepers represented in the current study migrated to Hokkaido Prefecture from all areas of Japan (Table 1). *Varroa destructor* K1-1/K1-2 might disseminate to other areas where the colonies once migrated. Moreover, *V. destructor* collected from apiaries located in Fukushima and Osaka Prefectures, which did not migrate to Hokkaido Prefecture, were also shown to be *V. destructor* K1-1/K1-2. *Varroa destructor* K1-1/K1-2 might rapidly spread throughout Japan. Information concerning *V. destructor* haplogroups present at other locations is lacking. Therefore, nationwide studies must be conducted to

determine the most prevalent *V. destructor* haplotypes to prevent further damage to *A. mellifera* colonies. Furthermore, global surveillance of *V. destructor* is required to identify the most highly infective *V. destructor* haplogroups.

In addition to *A. mellifera*, the current study investigated the dominant *V. destructor* haplogroups infesting *A. cerana* colonies in Japan. Although *A. cerana* is infested by *V. destructor*, they are resistant to *V. destructor* infestations (Boot et al. 1997; Fries et al. 1996). Surveillance of *V. destructor* in *A. cerana* colonies via sugar roll tests is not advisable because *A. cerana* colonies are quick to abscond when they experience any type of stress. Therefore, fewer numbers of *V. destructor* infesting *A. cerana* were investigated in this study. The dominant *V. destructor* haplogroup collected from *A. cerana* was *V. destructor* K1-1/K1-2. A single *V. destructor* collected at Matsue was determined to be *V. destructor* J1-3 (Fig. 1b). Navajas et al. (2010) collected *V. destructor* J1-2, 1–3, and 1–4 from *A. cerana* colonies in Tokyo, Machida, and Shikoku, respectively (Fig. 1a). Although the sample size was small, the genetic analysis of *V. destructor* in the current study indicated *V. destructor* K1-1/K1-2 infestation of *A. cerana* colonies (Fig. 1b). Loss of genetic variation may occur even in *A. cerana* colonies. The current results suggest that the spread of *V. destructor* K1-1/K1-2 to *A. cerana* colonies has already occurred probably due to the interactions between *A. cerana* and *A. mellifera*. Further analysis of the genetic structure of *V. destructor* using microsatellite DNA (Beaurepaire et al. 2015; Dynes et al. 2016; Roberts et al. 2015; Solignac et al. 2003, 2005) is necessary to understand the process of single haplogroup spread in Japan to prevent severe *V. destructor* infestations.

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