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Molecular evidence for multiple phylogenetic groups within two species of invasive spiny whiteflies and their parasitoid wasp

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Abstract

The invasive orange spiny whitefly (OSW) *Aleurocanthus spiniferus* has extended its distribution to non-native areas since the early 20th century. In a similar manner, the invasive tea spiny whitefly (TSW) *A. camelliae* has been expanding over East Asia in recent decades. In this study, the genetic diversity of OSW and TSW and of their important parasitoid wasp *Encarsia smithi* was investigated in China and Japan to enable more efficient biological control policies. We detected two phylogenetic groups (haplogroups A1 and A2) in OSW and three phylogenetic groups (haplotypes B1 and B2, and haplogroup B3) in TSW in China; however, only a single haplotype was detected in each whitefly species in Japan. Based on historical records and molecular data, OSW was considered to be native to China whereas TSW has probably expanded to China from a more southern location in the last 50 years; China appears to be the source region for OSW and TSW invading Japan. In *E. smithi*, two phylogenetic groups were detected in Japan: haplotype I, associated with OSW, and haplogroup II mostly associated with TSW, except in two locations. These data support the hypothesis that *E. smithi* parasitizing TSW in Japan did not originate from the existent population parasitizing OSW but was newly imported into Japan following the invasion of its host.

Keywords: *Aleurocanthus camelliae*, *Aleurocanthus spiniferus*, *Camellia sinensis*, citrus, *Encarsia smithi*, invasive pest

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Introduction

The introduction of agricultural pests into a new area has become common with the rapid globalization of agricultural products trade and increasing levels of tourism (Pimentel *et al.*, 2001). In most cases, because the new location is free from natural enemies, the population of introduced species rapidly increases (Torchin *et al.*, 2003). Introduced agricultural pests often lead to serious damage in agricultural crops and are therefore generally called invasive pests. Classical biological control

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methods (i.e., the intentional introduction of natural predators from the original home range of targeted invasive pests) have resulted in a reduction in pest densities in some cases (Bellows, 2001). One of the most successful examples of classical biological control is the introduction of the parasitoid wasp *Encarsia smithi* (Silvestri) (Hymenoptera: Aphelinidae) to control the orange spiny whitefly (OSW) *Aleurocanthus spiniferus* (Quaintance) (Homoptera: Aleyrodidae) (Nakao & Funasaki, 1979; Marutani & Muniappan, 1991; van den Berg & Greenland, 1997).

OSW is a relatively new invasive pests and known as one of the most destructive towards citrus plants, not only by generally weakening plants due to sap loss (direct damage) but also through the production of honeydew, which subsequently promotes the growth of sooty mould that covers the leaf surface and negatively affects photosynthesis (indirect damage) (Marutani & Muniappan, 1991; van den Berg & Greenland, 1997; Muniappan *et al*., 2006). OSW has a wide host range, including citrus, rose, grapevine, flowering apple, peach, and pear (Peterson, 1957; Nakao & Funasaki, 1979). Although OSW's original home range is assumed to be China and South and Southeast Asia, its distribution range has significantly extended to non-native areas such as Japan (Kuwana, 1934), the Caribbean islands (Gowdey, 1922), Micronesia (Peterson, 1955; Nafus, 1988; Muniappan *et al*., 1992, 2006), Hawaii (Nakao & Funasaki, 1979), southern Africa (van den Berg & Greenland, 1997; van den Berg *et al*., 2000), and Italy (Porcelli, 2008).

E. smithi is well-known as the main endoparasitoid wasp of OSW (Flanders, 1969); additionally, it can parasitize species of *Aleurocanthus* (Schauff *et al*., 1996; Heraty *et al*., 2007*b*; Dubey & Ko, 2012) and, in fact, an introduced population of *E. smithi* had the ability to control citrus blackfly *Aleurocanthu woglumi* Ashby populations in Mexico (Flanders, 1969). *E. smithi* reproduces sexually and asexually; both diploid females and haploid males can be generated by mated females parasitizing the spiny whitefly or by hyperparasitism on conspecific or congeneric larva, but only haploid males can be asexually produced by hyperparasitism (Nguyen & Sailer, 1987). *E. smithi* can kill spiny whiteflies not only by parasitizing it but also by the host-feeding behaviour (Kishida *et al*., 2010). In addition, *E. smithi* dispersal ability after introduction is high; in Micronesia islands, parasitism by this wasp was detected at distances over 1 km from release sites within a year after the release of only 24–67 individuals (Marutani & Muniappan, 1991). These ecological factors suggest *E. smithi* could be an effective agent for classical biological control.

Twenty *E. smithi* individuals collected in China were released in a citrus orchard in Japan in 1925 to control OSW (Kuwana, 1934). The wasp gradually established itself and spread to a considerable distance from the original release point (Kuwana, 1934). In addition, the wasp was bred and released in other areas affected by the whitefly, following an initiative of the Japanese government. Field pupal parasitism averaged 65% and sometimes exceeded 90% (Kuwana, 1934) and as a result, OSW has been almost completely eliminated, except for occasional outbreaks in limited regions (Ohgushi, 1969). Similar introductions were performed in Micronesia (Nafus, 1988; Marutani & Muniappan, 1991; Muniappan *et al*., 1992), Hawaii (Nakao & Funasaki, 1979), and southern Africa (van den Berg & Greenland, 1997). Within a year of release, it had almost eradicated OSW, eliminating the need for pesticide applications (e.g., Marutani & Muniappan, 1991; van den Berg & Greenland, 1997).

In recent decades, the tea spiny whitefly (TSW) *Aleurocanthus camelliae* (Kanmiya & Kasai) has affected tea production in East Asia. This species is phylogenetically close to OSW and until the recent publication of detailed ecological, morphological, and molecular phylogenetic studies the two species were considered as a single species (Kanmiya *et al*., 2011). The damages caused by TSW include the reduction in tree vigour, caused by sap loss and sooty mould, and the reduction in harvesting efficiency due to flying adults disturbing farmworkers in tea plantations (Yamashita & Hayashida, 2006). Known hosts of TSW are Theaceae plants including *Camellia sinensis* (L.) Kuntze, *Camellia sasanqua* Thunb., *Cleyera japonica* Thunb., *Eurya japonica* Thunb., and *Illicium anisatum* L., which does not overlap with OSW hosts. Kasai *et al*. (2010) recognized significant differences in host preference between OSW and TSW based on oviposition and larval feeding behaviour: OSW laid no eggs on *Camellia* leaves; TSW laid a few eggs on *Citrus* but no nymphs settled on the leaves. Therefore, the tea-infesting spiny whitefly, which had been previously described as OSW, should be identified as TSW (Uesugi & Sato, 2011).

Damage to tea plantations due to TSW was initially observed in southern China in the 1950s and became serious in the 1980s (Xie, 1995; Xuefen *et al*., 1997; Han & Cui, 2003). Subsequently, TSW also became an important pest in Taiwan (Hsiao & Shiau, 2004) and it was initially identified in Kyoto, Japan, in 2004 (Yamashita & Hayashida, 2006). Although there was no intentional introduction of *E. smithi* in Japan, a high rate of parasitism by this wasp stabilized the whitefly population in some regions below the economic injury level (Yamashita, personal communication). However, the wasp has not become established in some of the regions invaded by TSW (e.g., Yakushima Island).

The origin of *E. smithi* parasitizing TSW in Japan is unclear. One hypothesis is that wasps from an existing population parasitizing OSW began to parasitize TSW; another advocates the wasp has immigrated to Japan following the invasion of TSW and is phylogenetically distinct from the existing population that parasitizes OSW. In the latter case, we should consider the risk of undesirable side effects, referred to as 'non-target' effects, associated with the use of the newly imported genetic group of *E. smithi* parasitizing TSW as a biological control agent (Follet & Duan, 2000).

The purpose of this study was to investigate the genetic diversity of OSW and TSW, and their important natural enemy, *E. smithi*, to provide information for the implementation of biological control by the wasp. Molecular markers have proven to be useful for distinguishing cryptic diversity in whiteflies and in Aphelinidae wasps at the species and population levels (Hoy *et al*., 2000; Rajaei Shoorcheh *et al*., 2008; De Leon *et al*., 2010). They are also useful for determining both the source area and population structure of invasive species (Roderick & Navajas, 2003; Cognato *et al*., 2005; Simon-Bouhet *et al*., 2006; Kirk *et al*., 2013) and for providing evidence to determine whether single or multiple sources of introduction occurred (Grapputo *et al*., 2005). In this study, we first investigated the genetic variation within the two species of spiny whiteflies and estimated their potential native area using a sequence fragment of the cytochrome C oxidase subunit I of mitochondrial DNA (mtCOI). We then investigated genetic variation through the characterization of mtCOI haplotypes and studied the genetic relationships among *E. smithi*, OSW, and TSW. Finally, we evaluated the genetic diversity of *E. smithi* using microsatellites to unveil the recent demographic events shaping the wasp's colonization after its introduction in Japan.

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Materials and methods

Sample collection

OSW and TSW adults and nymphs were collected from April 2008 to January 2012 in Japan and China (table 1, figs 1 and 2). OSW samples were collected from 21 localities in 11 prefectures (provinces) and TSW samples from 47 localities in 26 prefectures (provinces) (table 1). In Japan, TSW was initially identified in Uji city, Kyoto (KYO2) (table 1, fig. 1) in 2004 (Yamashita & Hayashida, 2006), later expanding to neighbour regions within the Kyoto Prefecture (KYO3–KYO8), and to the prefectures of Mie (MIE2–MIE5), Nara (NAR1–NAR3), Osaka (OSA), Hyogo (HYG), Shiga (SHG), and Gifu (GIF) (table 1 and fig. 1). The cause for this expansion was attributed to host plants movement (Kasai *et al*., 2010). OSW was collected from four species of *Citrus* (*Citrus unshiu* Markobich, *Citrus hassaku* Hort. Ex Tanaka, *Citrus depressa* Hayata, *Citrus keraji* var. kabuchii hort. ex Tanaka) and one Fabaceae (*Caesalpinia crista* L.). TSW was collected from four Theaceae (*C. sinensis* (L.), *C. sasanqua*, *E. japonica*, and *C. japonica*), one Illiciaceae (*I. anisatum*), and one Rutaceae (*Zanthoxylum piperitum* DC.) (table 1). Six TSW nymphs were collected from *E. japonica* and *C. japonica* intercepted in Japanese quarantine after being imported from China (table 1, fig. 2). All samples were stored in 99.5% ethanol at −20°C until genetic analysis.

Samples of the parasitoid wasp *E. smithi* were obtained from leaves infected with OSW and TSW nymphs collected from 15 localities in seven prefectures and from nine localities in eight prefectures, respectively. Leaves were collected from May 2005 to September 2012 (table 2, fig. 3) and adult female wasps that emerged from the nymphs were placed in clear plastic boxes. Wasps were morphologically identified to the species level (Schauff *et al*., 1996), and stored in 99.5% ethanol at −20°C until genetic analysis. Additionally, 21 individuals from six localities (Shizuoka, Ehime, Okinawa, Gifu, Mie, and Nara; table 2) were used to confirm species identification through the analysis of a partial nucleotide sequence of the D2 expansion region of the large subunit of ribosomal DNA (28S rDNA), which was suggested as the most suitable genetic region for taxonomic studies at species level in *Encarsia* spp. (Schmidt *et al*., 2011). Up to eight individuals collected in each locality were used in the mtCOI sequence analysis and up to 32 individuals from ten localities were used for microsatellite analysis (table 2).

DNA extraction and polymerase chain reaction (PCR)

Total DNA was extracted from individual whiteflies (adults and nymphs) and wasps (adult females) according to the proteinase K method (Goka *et al*., 2001).

To confirm *E. smithi* morphological identification, the partial nucleotide sequence of D2 was obtained by PCR amplification using the primers D2F (5′-CGG GTT GCT TGA GAG TGC AGC-3′, forward) and D2Ra (5′-CTC CTT GGT CCG TGT TTC-3′, reverse) (Schmidt *et al*. 2011). PCR was performed with a GeneAmp PCR System Model 9700 (Applied Biosystems, Foster City, CA, USA) in a total volume of 20 µl containing 0.5 µM of each primer, 0.2 mM of dNTP, 1 × Ex Taq buffer (2 mM $MgCl_2$), 1 U of Ex Taq DNA polymerase (TaKaRa, Shiga, Japan), and 1 µl of DNA template. A negative control (de-ionized water instead of DNA template) was added to each reaction to confirm there was no contamination. The amplification profile used was: 5 min at 96°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 90 s at 72°C, and a final extension set of 5 min at 72°C.

PCR was also used to obtain the partial mtCOI nucleotide sequence using the following primers for each species: OSW: 5′-GTG TCC CAT TTA ATT AGT AGA GA-3′ (forward) and 5′-GAG CCA TAA TAA AAG ACT CCA TC-3′ (reverse) (Uesugi & Sato, 2011); TSW: 5′-ATT TCA CAC TTA ATT AGG AGT GA-3′ (forward) and 5′-CTG CAC GAA ATA CAA CAA ATG C-3′ (reverse) (Uesugi & Sato, 2011); *E. smithi*: 5′-TTG ATT TTT TGG TCA TCC AGA AGT-3′ (C1-J-2195, forward) and 5′-TCC AAT GCA CTA ATC TGC CAT ATT A-3′ (L2-N-3014, reverse) (Simon *et al*., 1994). All PCR mixtures were identical to the one used in the amplification of the D2 region of *E. smithi* as described above. A negative control (de-ionized water instead of DNA template) was also added to each of these reactions to confirm there was no contamination. The PCR profile used was: 5 min at 96°C, followed by 35 cycles of 30 s at 94°C, 1 min at 53°C, and 40 s at 72°C, with a final elongation step of 5 min at 72°C. PCR products were subjected to an agarose gel electrophoresis and those revealing a single band of the predicted size (ca. 900 bp) were purified by ExoSAP-IT (Affymetrix, Santa Clara, CA, USA). Sequencing was performed on an ABI 310 genetic analyzer (Applied Biosystems) using the ABI BigDye v.3.1 (Applied Biosystems) according to the manufacturers' recommendations and using the same primers as in PCR.

Five microsatellite loci were amplified by PCR from individuals belonging to 10 populations of the parasitoid wasp using the primer pairs Es002, Es011, Es051, Es060, and Es083 and the profiles described by Uesugi & Sato (2013). PCR products were analyzed on an ABI 310 genetic analyzer (Applied Biosystems) and GeneMapper (Applied Biosystems) was used to identify the microsatellite genotypes within each sample.

Data analyses

All sequence data obtained in the present study were deposited in the DNA Data Bank of Japan (DDBJ) and their accession numbers are referred throughout the text. The D2 sequences obtained for *E. smithi* (589 bp) were checked for homology with *E. smithi* D2 sequences deposited in the DDBJ using the Basic Local Analysis Search Tool (BLAST, last accessed date: 3 October 2015) in order to confirm species morphological identification.

The 674 bp partial mtCOI sequences obtained for OSW and TSW were aligned in CLUSTAL W (Thompson *et al*., 1994) using the default parameters and examined for variation. No insertions/deletions were found. A median-joining (MJ) network (Bandelt *et al*., 1999) was constructed for this gene in Network 4.611 (Fluxus Technology, Suffolk, UK) using the default settings. The best model of nucleotide substitution was selected using jModelTest 2.1.7 (Guindon & Gascuel, 2003; Darriba *et al*., 2012). Based on the Bayesian Information Criterion (BIC) results, the Hasegawa-Kishino-Yano (HKY) + Gamma distributed rates (G) model was selected for phylogenetic analyses. Trees were constructed using the maximum-likelihood (ML) and Bayesian inference (BI) algorithms in MEGA 6.06 (Tamura *et al*., 2013) and MrBayes 3.2 (Ronquist *et al*., 2012), respectively. A mtCOI sequence of *Aleurotrachelus camelliae* Kuwana (Accession No. AB536801) (Kanmiya *et al*., 2011) was used as outgroup. Bootstrapping with 1000 replicates and posterior probabilities assessed the degree of confidence assigned to nodes in ML and BI trees, respectively. A species delimitation analysis was performed by

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Table 1. Sampling location (including code) and year, host plant, and number (*n*) of *Aleurocanthus spiniferus* (orange spiny whitefly, OSW) and *A. camelliae* (tea spiny whitefly, TSW) individuals used in the mtCOI sequence analysis and their corresponding phylogenetic group.

Code	City	Prefecture or Province	Country	Year	Host plant	*n*	Phylogenetic group
OSW							
KAN	Matsuda	Kanagawa	Japan	2009	*Citrus unshiu*	1	A1
SHZ1	Shimizu	Shizuoka	Japan	2010	*C. unshiu*	4	A1
SHZ2	Okitsu	Shizuoka	Japan	2010	*C. unshiu*	4	A1
SHZ3	Fujieda	Shizuoka	Japan	2010	*C. unshiu*	4	A1
MIE1	Suzuka	Mie	Japan	2010	*Citrus hassaku*	4	A1
KYO1	Miyazu	Kyoto	Japan	2008	*C. unshiu*	4	A1
EHI	Seiyo	Ehime	Japan	2010	*C. unshiu*	4	A1
OIT1	Kitsuki	Oita	Japan	2009	*C. unshiu*	4	A1
KGS	Kagoshima	Kagoshima	Japan	2010	*C. unshiu*	4	A1
AMA1	Amami (Tatsugo)	Kagoshima	Japan	2010	*C. unshiu*	4	A1
AMA2	Amami (Naze)	Kagoshima	Japan	2010	*Caesalpinia crista*	4	A1
OKI1	Ogimi	Okinawa	Japan	2011	*Citrus depressa*	4	A1
OKI2	Kunigami	Okinawa	Japan	2011	*Citrus keraji*	4	A1
OKI3	Gesashi	Okinawa	Japan	2011	*C. crista*	4	A1
ISG1	Ishigaki	Okinawa	Japan	2010	*C. depressa*	1	A1
ISG2	Ishigaki	Okinawa	Japan	2010	*C. crista*	4	A1
IRI1	Iriomote	Okinawa	Japan	2010	*C. depressa*	1	A1
IRI2	Iriomote	Okinawa	Japan	2010	*C. crista*	4	A1
ZHE1	Taizhou	Zhejiang	China	2010	*C. unshiu*	12	A1,A2
HUB	Yichang	Hubei	China	2010	*C. unshiu*	12	A1,A2
GUA1	Qingyuan	Guangdong	China	2011	*C. unshiu*	12	A1
					Total	99	
TSW							
STM	Sayama	Saitama	Japan	2009	*Camellia sinensis*	4	B1
TKY	Mizuho	Tokyo	Japan	2011	*C. sinensis*	4	B1
SHZ4	Numazu	Shizuoka	Japan	2011	*C. sinensis*	4	B1
SHZ5	Shizuoka	Shizuoka	Japan	2011	*C. sinensis*	4	B1
SHZ6	Kikugawa	Shizuoka	Japan	2010	*C. sinensis*	4	B1
AIC	Inazawa	Aichi	Japan	2010	*C. sasanqua*	4	B1
GIF	Ogaki	Gifu	Japan	2010	*C. sinensis*	4	B1
SHG1	Koga	Shiga	Japan	2009	*C. sinensis*	4	B1
SHG2	Koga	Shiga	Japan	2010	*Zanthoxylum piperitum*	4	B1
MIE2	Kameyama	Mie	Japan	2008	*C. sinensis*	4	B1
MIE3	Odai	Mie	Japan	2008	*C. sinensis*	4	B1
MIE4	Matsuzaka	Mie	Japan	2008	*C. sinensis*	4	B1
MIE5	Kameyama	Mie	Japan	2010	*Z. piperitum*	4	B1
FKI	Obama	Fukui	Japan	2010	*C. sinensis*	4	B1
KYO2	Uji	Kyoto	Japan	2008	*C. sinensis*	4	B1
KYO3	Kyotanabe	Kyoto	Japan	2008	*C. sinensis*	4	B1
KYO4	Wazuka	Kyoto	Japan	2008	*C. sinensis*	4	B1
KYO5	Tamba	Kyoto	Japan	2008	*C. sinensis*	4	B1
KYO6	Ujitawara	Kyoto	Japan	2008	*C. sinensis*	4	B1
KYO7	Saigyo	Kyoto	Japan	2009	*C. sasanqua*	4	B1
KYO8	Wazuka	Kyoto	Japan	2008	*Eurya japonica*	4	B1
NAR1	Nara	Nara	Japan	2009	*C. sinensis*	4	B1
NAR2	Yamatokoriyama	Nara	Japan	2009	*C. sinensis*	4	B1
NAR3	Yamazoe	Nara	Japan	2009	*C. sinensis*	4	B1
OSA	Toyonaka	Osaka	Japan	2010	*C. sinensis*	4	B1
HYG	Sasayama	Hyogo	Japan	2010	*C. sinensis*	4	B1
OKA	Maniwa	Okayama	Japan	2010	*C. sinensis*	4	B1
SHM1	Misato	Shimane	Japan	2009	*C. sinensis*	4	B1
SHM2	Matsue	Shimane	Japan	2009	*C. sinensis*	4	B1
SHM3	Tsuwano	Shimane	Japan	2010	*C. sinensis*	4	B1
SHM4	Misato	Shimane	Japan	2009	*Illicium anisatum*	4	B1
FKO2	Yame	Fukuoka	Japan	2009	*C. sinensis*	4	B1
KUM	Kahoku	Kumamoto	Japan	2012	*C. sinensis*	4	B1
OIT2	Kitsuki	Oita	Japan	2009	*C. sinensis*	4	B1
YAK	Yakushima	Kagosim	Japan	2012	*C. sinensis*	4	B1
ANH	Xuancheng	Anhui	China	2008	*C. sinensis*	12	B1
ZHE2	Jinhua	Zhejiang	China	2008	*C. sinensis*	12	B1
ZHE3	Ningde	Zhejiang	China	2011	*C. sinensis*	2	B1
ZHE4	Wenzhou	Zhejiang	China	2011	*C. sinensis*	7	B1
FUJ	Fu'an	Fujian	China	2008	*C. sinensis*	12	B1

Continued

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Table 1. (*Cont*.)

Code	City	Prefecture or Province	Country	Year	Host plant	*n*	Phylogenetic group
GUA2	Yingde	Guangdong	China	2008	*C. sinensis*	12	B1
GUA3	Qingyuan	Guangdong	China	2011	*C. sinensis*	3	B1
JIA	Jiujiang	Jiangxi	China	2011	*C. sinensis*	12	B1
HUN	Changsha	Hunan	China	2008	*C. sinensis*	12	B1
SIC	Chengdu	Sichuan	China	2008	*C. sinensis*	12	B1
CHO1	Yongchuan	Chongqing	China	2008	*C. sinensis*	12	B2
CHO2	Yongchuan	Chongqing	China	2011	*C. sinensis*	12	B2
J-QUA1	Intercepted in Japanese Quarantine		China	2010	*E. japonica*	3	B1,B3
J-QUA2	Intercepted in Japanese Quarantine		China	2011	*E. japonica*	1	B1
J-QUA3	Intercepted in Japanese Quarantine		China	2011	*Cleyera japonica*	2	B3
					Total	266	

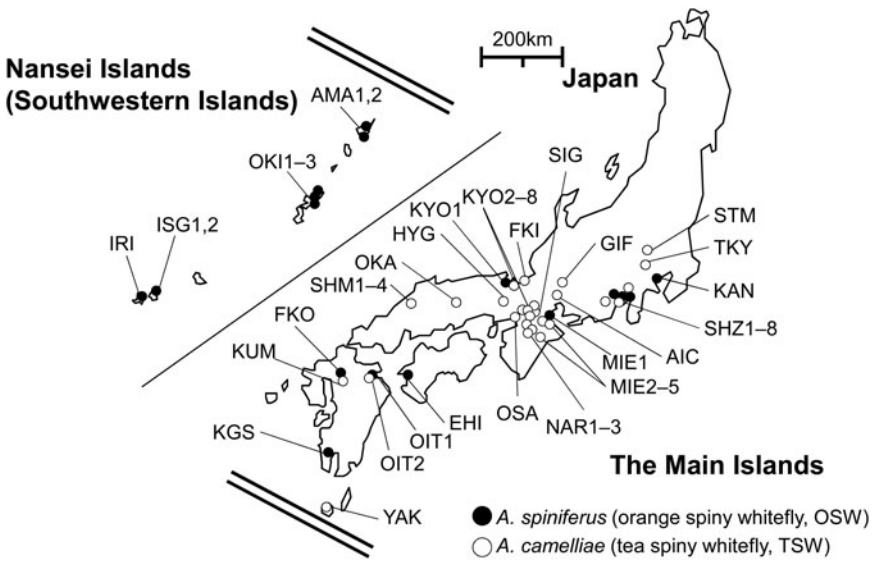

Fig. 1. Collection sites of the orange spiny whitefly and tea spiny whitefly individuals in Japan.

the Poisson-Tree-Process (PTP) on the bPTP web server of the Exelixis Lab (http://species.h-its.org/ptp/) using the default settings and a Bayesian implementation of the model (Zhang *et al*., 2013), and based on ML and BI trees. Haplotype diversity (*h*: Nei & Tajima, 1981) was calculated for OSW and TSW, and for each putative phylogenetic group detected by the network analysis and the phylogenetic trees, as $h = (1 - \Sigma x_i^2)\, n/(n-1)$, where Σx_i is the frequency of the *i*th haplotype and *n* is the number of samples.

Evaluation of the variation of *E. smithi* mtCOI sequences (734 bp) and construction of ML and BI phylogenetic trees were performed as described for the whiteflies. A General Time Reversible (GTR) model + percentage of Invariable sites (I) model was selected by jModelTest 2.1.7. mtCOI sequences of *Encarsia formosa* Gahan (Accession No. AY264337) (Heraty *et al*., 2007*a*) and *Marietta montana* Myartseva and Ruíz-Cancino (Accession No. DQ350502) were used as outgroups.

Microsatellite data of *E. smithi* was checked for scoring errors and null alleles in each locus and within each population using Micro-Checker 2.2.3 (Brookfield, 1996; van Oosterhout *et al*., 2004). The number of alleles and expected heterozygosity of *E. smithi* were determined for each population using the five loci. FSTAT 2.9.3.2 (Goudet, 2001) was used to detect genotypic disequilibrium among microsatellite loci and to quantify the level of genetic differentiation (θ) among wasp populations within each host species. The 95% confidence intervals for θ were calculated from 1000 permutations of the genotypes. Pairwise genetic distances among wasp individuals were calculated using the method described by Smouse & Peakall (1999) using GENALEX 6 (Peakall & Smouse, 2006). We then evaluated the genetic structure of the populations by performing a principal coordinates analysis (PCA) in GENALEX. To assess migration among populations of the wasp, we applied coalescent-based Markov Chain Monte Carlo (MCMC) simulations using the ML analysis implemented in Migrate-N 3.6 (Beerli, 2006). Migrate-N estimates mutation-scaled effective population size (Θ; $\Theta = 4N_e\mu$, where N_e is the effective population size and μ is the mutation rate per generation) in each population as well as mutation-scaled migration rates (M; $M = m/\mu$, where m is the migration rate) between population pairs. We calculated the number of

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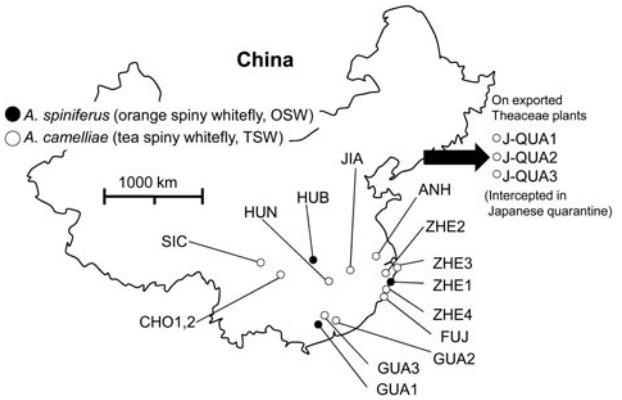

Fig. 2. Collection sites of the orange spiny whitefly and tea spiny whitefly individuals in China.

effective migrants per generation (N_em; $N_em = \Theta M/4$) using the values estimated by Migrate-N. Simulations used ten short chains of 2×10^4 steps and two long chains of 2×10^5 steps, sampled every 20 steps following a burn-in of 1000 steps. To increase the efficiency of the MCMC, a heating scheme was applied using four chains with temperatures 1.00, 1.50, 3.00, and 1.00×10^6. Default settings were used in the remaining run parameters.

Genetic structure was investigated through the Bayesian clustering method implemented in Structure 2.3 (Pritchard *et al.*, 2000). We used a burn-in period of 1×10^4 iterations and a MCMC run length of 1×10^4 iterations. Ten runs were carried out for each K ($K = 1$ to 10 clusters) in order to quantify the amount of variation of the likelihood. K was determined in Structure Harvester 0.6.9.3 (Evanno *et al.*, 2005), which uses an ad hoc parameter (ΔK) to estimate the rate of change of likelihood values between successive Ks.

Results

Genetic variation and haplotype distribution of OSW and TSW

Mitochondrial COI sequence analyses revealed eight haplotypes in the 36 OSW individuals obtained from the three localities in China; in Japan, a single haplotype was found for the 63 OSW individuals infesting *Citrus* spp. and *C. crista* host plants in the 18 localities. Network analysis revealed two discontinuous phylogenetic groups separated by 75 mutations (fig. 4), and these two groups were also evidenced in the ML and BI phylogenetic trees (fig. 5a; only the ML tree is shown). The two phylogenetic groups, named A1 (Accession No. AB786715–AB786717) and A2 (Accession No. AB786718–AB786723) (table 1, figs 4 and 5a) were found in sympatry in the Chinese populations ZHE1 and HUB. Haplotype diversity (h) in A2 (0.671) was higher than in A1 (0.420) and moderately high in OSW (0.604) (fig. 4).

In TSW, two haplotypes were detected in the 120 individuals from the 11 tea plantations in China; one was named haplotype B1 (Accession No. LC088497) and the other was named haplotype B2 (Accession No. AB786712) (table 1). Haplotype B1 was detected across a wide range in China, but haplotype B2 was only detected in Chongqing (populations CHO1 and CHO2). In Japan, a single mtCOI haplotype (B1) was observed for the 140 individuals from the 35 localities infecting any host plant (table 1). The individuals intercepted in the Japanese quarantine had three haplotypes: B1, and B3-1 and B3-2 (Accession No. AB786713–AB786714), which only differed by one-base substitution (table 1). Network analysis (fig. 4) revealed three phylogenetic groups within TSW corresponding to the three haplotypes (fig. 4). Phylogenetic analyses also revealed B1 and B3 were more closely related than B1 and B2 (fig. 5a). B1 and B2 showed no haplotype diversity ($h = 0$), whereas haplogroup B3 had a moderately high h (0.600); total haplotype diversity within TSW was low (0.199) (fig. 4).

Regarding the bPTP molecular species delimitation analyses, A1 and A2 were recognized as different putative species with high posterior probabilities in the ML (0.898 and 0.949, respectively) and BI (0.981 and 0.905, respectively) trees. B1, B2, and B3 were also recognized as different putative species with high posterior probabilities in both trees (0.989, 0.989, and 0.959 in the ML tree, and 1.00, 1.00, and 0.999 in the BI tree, respectively).

Genetic variation and phylogeny of *E. smithi*

We confirmed the species identification of *E. smithi* using the sequence data of D2. In the nine samples collected from OSW in Japan, a single diplotype (Accession No. LC088727) was detected. The sequence was identical to samples of *E. smithi* from Hawaii deposited in the DNA Data Bank of Japan (Accession No. AF254233) (Babcock *et al.*, 2001). In the 12 samples parasitizing TSW in Japan, three diplotypes (Accession No. LC088728–LC088730) were detected, which were highly similar to *E. smithi* from the Truk Islands, Micronesia (Accession No. AF254234) (Babcock *et al.*, 2001). Rate of nucleotide substitution between *E. smithi* D2 haplogroups I and II was 1.86–2.04% (11–12 substitutions per 589 bp).

A single haplotype was found for the mtCOI of the 107 *E. smithi* individuals parasitizing OSW in 15 localities in Japan (haplotype I, Accession No. AB786726) (table 2). Fifty-five *E. smithi* individuals parasitizing TSW in seven localities also showed a single haplotype (Accession No. AB786724), and one sample (population HYG) had an analogous haplotype with only one base substitution (Accession No. AB786725). These were named haplogroup II (table 2). Wasps of populations SHZ5 and FKO2 parasitizing TSW were identified within haplotype I (table 2). Rate of nucleotide substitution between mtCOI haplogroups I and II was 7.35% (54 substitutions per 734 bp). This differentiation was also evidenced in the ML phylogenetic tree of *E. smithi* displayed in fig. 5b, which showed a high bootstrap support for the node between haplotype I and haplogroup II (99%). A high posterior probability (1.00) was also obtained in the Bayesian tree (data not shown).

Microsatellite polymorphisms in E. smithi

Null alleles and possible stuttering in all loci and populations were not significant ($P > 0.95$), except in population FKO1 where null alleles might be present at loci Es002 and Es051, as suggested by the general excess of homozygotes for most alleles size classes ($P < 0.05$). In addition, significant genotypic disequilibrium was not detected between any pair of microsatellite loci within the phylogenetic groups ($P > 0.05$).

Indices of genetic diversity and differentiation of *E. smithi* are shown in table 3. Genetic differentiation (θ) among populations was significantly positive for populations within the same host (table 3). In haplotype I, the genetic diversity of

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Table 2. Sampling location (including code) and year, host (orange spiny whitefly, OSW, and tea spiny whitefly, TSW), host plant, and number (*n*) of *E. smithi* used in the mtCOI, 28S rDNA, and microsatellites analyses.

Code	City or Residency	Prefecture	Year	Host	Host plant	mtCOI	28S rDNA	Microsatellites	Phylogenetic group
SHZ1	Shimizu	Shizuoka	2010	OSW	*Citrus unshiu*	8	–	32	I
SHZ2	Okitsu	Shizuoka	2010	OSW	*C. unshiu*	8	3	32	I
SHZ3	Fujieda	Shizuoka	2010	OSW	*C. unshiu*	8	–	–	I
KYO1	Miyazu	Kyoto	2008	OSW	*C. unshiu*	8	–	–	I
EHI	Seiyo	Ehime	2010	OSW	*C. unshiu*	8	3	32	I
FKO1	Chikugo	Fukuoka	2009	OSW	*C. unshiu*	8	–	12	I
KUM	Kumamoto	Kumamoto	2010	OSW	*C. unshiu*	2	–	–	I
AMA1	Amami (Naze)	Kagoshima	2010	OSW	*C. unshiu*	8	–	32	I
AMA2	Amami (Tatsugo)	Kagoshima	2010	OSW	*C. unshiu*	8	–	–	I
AMA3	Amami	Kagoshima	2010	OSW	*C. unshiu*	8	–	–	I
OKI	Gesashi	Okinawa	2011	OSW	*Caesalpinia crista*	8	3	32	I
ISG1	Ishigaki (Nosoko)	Okinawa	2010	OSW	*Citrus depressa*	1	–	–	I
ISG2	Ishigaki (Nagura)	Okinawa	2010	OSW	*C. crista*	8	–	–	I
IRI1	Iriomote (Komi)	Okinawa	2010	OSW	*C. depressa*	8	–	32	I
IRI2	Iriomote (Sonai)	Okinawa	2010	OSW	*C. crista*	8	–	–	I
SHZ4	Shizuoka	Shizuoka	2011	TSW	*Camellia sinensis*	8	–	–	II
SHZ5	Numazu	Shizuoka	2011	TSW	*C. sinensis*	8	–	–	I
GIF	Ogaki	Gifu	2010	TSW	*C. sinensis*	8	4	32	II
MIE	Kameyama	Mie	2009	TSW	*C. sinensis*	8	4	32	II
SHG	Koga	Shiga	2009	TSW	*C. sinensis*	8	–	–	II
KYO2	Uji	Kyoto	2005	TSW	*C. sinensis*	8	–	32	II
NAR	Nara	Nara	2010	TSW	*C. sinensis*	8	4	–	II
HYG	Sasayama	Hyogo	2010	TSW	*C. sinensis*	8	–	–	II
FKO2	Yame	Fukuoka	2012	TSW	*C. sinensis*	8	–	–	I
					Total	179	23	300	

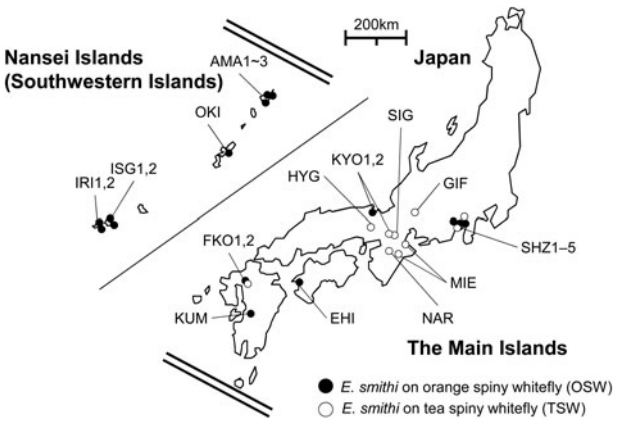

Fig. 3. Collection sites of *Encarsia smithi* individuals in Japan.

microsatellites was different between populations from the main islands (SHZ1, SHZ2, EHI, and FKO1) and Nansei islands (AMA1, OKI, and IRI1). Populations from the main islands had relatively high heterozygosities and allelic diversity (table 3) and were clustered into a single group in the PCA (fig. 6); populations from the Nansei islands had low heterozygosities and allelic diversity (table 3) and appeared widely scattered in the PCA, also indicating that these populations had a higher level of genetic differentiation than populations from the main islands. Haplogroup II had low heterozygosity and allelic diversity, and a high level of genetic differentiation as revealed by the scattering of populations in the PCA diagram (fig. 6).

ML-based analysis using the program Migrate-N showed that gene flow was low among wasp populations. When *M* values were translated into effective migrants per generation (N_em), the numbers of effective migrants per generation were below 1 in most population pairs (table 4), suggesting that either migration rate (*m*) or the effective population size (N_e) decreased due to population division and/or genetic drift (Slatkin, 1985).

In the Bayesian clustering analysis using the Structure, the likelihood of individuals assignment increased from $K = 1$ (ln Pr (X | K) = −2365) to $K = 5$ (ln Pr (X | K) = −1363), and then plateaued until $K = 10$ (ln Pr (X | K) = −1313). Therefore, the highest likelihood was estimated to be at $K = 5$. Structure harvester showed that the highest values for the estimated likelihood of *K* were found at $K = 2$ and $K = 5$ ($\Delta K = 236.75$, 3.82, 3.99, *18.78*, 0.65, 0.16, 0.29, and 1.20 from $K = 2$ to $K = 9$, respectively). In $K = 2$, individuals within the SHZ1, SHZ2, EHI, FKO1, and AMA1 were almost all classified in the same cluster (fig. 7), which is in agreement with that obtained in the PCA analysis (fig. 6). In $K = 5$, individuals within the SHZ1, SHI2, EHI, and FKO1 populations were mostly classified into two or three clusters (fig. 7), although the assignment profiles largely differed between individuals within the same population. Population AMA1 and populations OKI and IRI1 mostly consisted of a single cluster with most individuals being assigned to the clusters; populations GIF, MIE, and KYO2 also mostly consisted of a single cluster, which was different from the ones detected in the wasps parasitizing OSW (fig. 7).

Discussion

Phylogeny and haplotype diversity of the OSW

Native populations of invasive species generally preserve higher levels of genetic diversity than introduced populations (Malacrida *et al*., 1998; Bonizzoni *et al*., 2004; Dlugosch & Parker, 2008). The reduction in mitochondrial genetic diversity observed in introduced populations has been attributed to genetic bottlenecks originated by the recent human-aided

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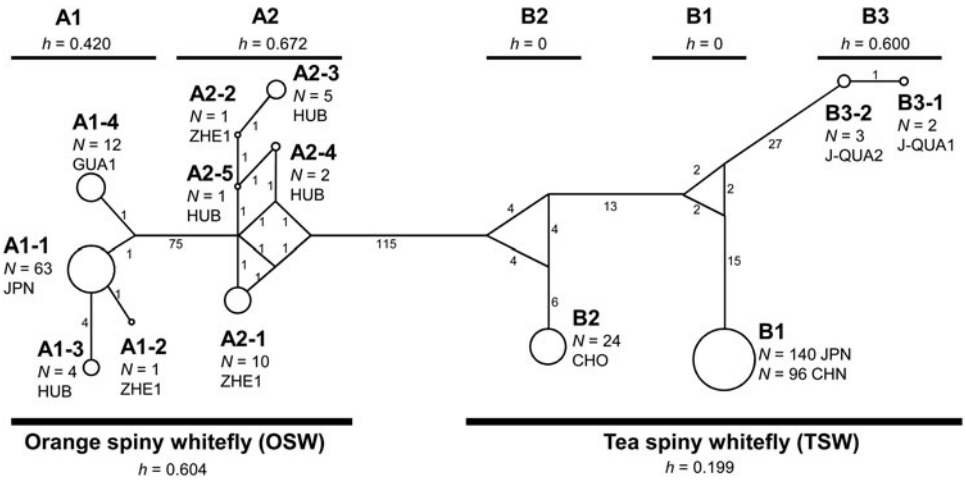

Fig. 4. Median-joining (MJ) network based on the mtCOI sequences of the orange spiny whitefly (OSW) and tea spiny whitefly (TSW), in Japan (JPN) and China (CHN). A1 and A2, and B1, B2, and B3 are the putative phylogenetic groups obtained for OSW and TSW, respectively. *N* corresponds to the detected number of each haplotype. Collection site codes are described in Table 1. Haplotype diversities (*h*, Nei & Tajima, 1981) are shown for each species and for each phylogenetic group. Numbers of mutations between haplotypes are shown next to the lines.

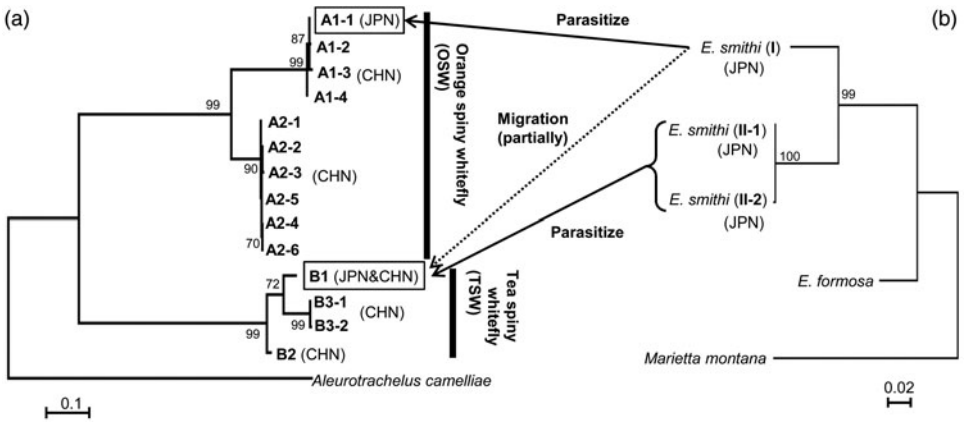

Fig. 5. Maximum likelihood trees based on mtCOI sequence data showing (a) the relationships between orange spiny whitefly and tea spiny whitefly phylogenetic groups from Japan and China, and (b) the relationships between the phylogenetic groups of the parasitoid wasp *Encarsia smithi* from Japan. Only bootstrap values above 70% are shown; scale bars indicate nucleotide substitutions per site.

expansions, which caused stochastic lineage survival and reduced the number of haplotypes (Kambhampati & Rai, 1991; Guillemaud *et al.*, 1997). Mitochondrial COI haplotype diversity in OSW populations from China was higher than that in populations from Japan. Therefore, China seems to be the source area of OSW with Japan being only recently invaded. In addition, this invasion was most likely a unique event, as multiple invasions would have resulted in several haplotypes (Grapputo *et al.*, 2005). This is in agreement with the suggestion in old literature that OSW immigrated to Japan from somewhere south of the region based on OSW not being recognized in Japan before its first outbreak in the early 1900s (Kuwana, 1934).

Phylogeny and haplotype diversity of TSW

All populations of TSW in Japan and most populations in China had a single fixed haplotype in mtCOI (haplotype B1), contrasting with the levels of genetic variation found for the Chinese populations of OSW. One explanation for this difference is the recent expansion of TSW to tea plantations in China from an unknown native region during which populations experienced a strong genetic bottleneck. This recent expansion is in agreement with field observations of whiteflies in China's tea plantations (Xie, 1995; Xuefen *et al.*, 1997; Han & Cui, 2003). Haplotype B2, which was only detected in the populations of Chongqing (CHO1 and CHO2), might be native to this region or have immigrated during a different invasion event, as the molecular species delimitation analyses evidenced an indisputably different phylogenetic history for B1 and B2.

Because haplotype B1 was detected in whiteflies infecting hosts in Japanese quarantine, the invasion pathway from China to Japan was probably the international trade of *E. japonica* and *C. japonica*. Because these plants are used in religious ceremonies held in Japanese shrines, TSW individuals

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Table 3. Genetic diversity and differentiation of *Encarsia smithi* parasitizing the orange spiny whitefly (OSW) and the tea spiny whitefly (TSW) in Japan based on five microsatellites loci.

Population	Host	Phylogenetic group	*N*	Mean *NA*	Polymorphic loci	Mean H_S	θ
SHZ1	OSW	I	32	3.40	5	0.425	0.556 (0.453–0.641)
SHZ2	OSW	I	32	3.00	5	0.401	
EHI	OSW	I	32	3.20	4	0.366	
FKO1	OSW	I	12	3.00	5	0.456	
AMA1	OSW	I	32	1.20	1	0.0776	
OKI	OSW	I	32	1.20	1	0.0240	
IRI1	OSW	I	32	1.00	0	0.000	
GIF	TSW	II	32	1.40	2	0.142	0.561 (0.139–0.852)
MIE	TSW	II	32	1.60	2	0.165	
KYO2	TSW	II	32	1.60	1	0.0328	

NA, number of alleles; H_S, heterozygosity; θ, genetic differentiation; numbers in parentheses indicate 95% confidence intervals.

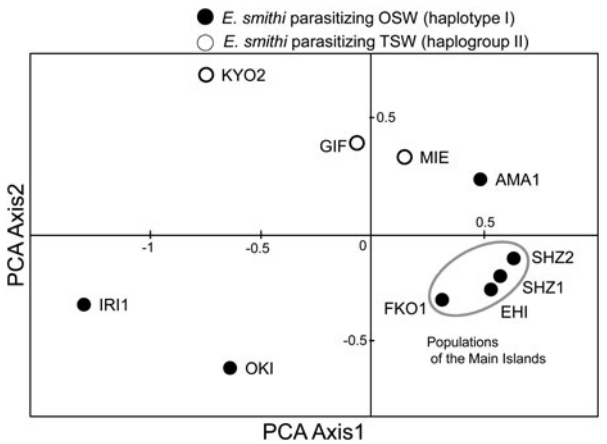

Fig. 6. Principal coordinates analysis (PCA) of the genetic variation among the populations of *Encarsia smithi* parasitizing the orange spiny whitefly (OSW) and the tea spiny whitefly (TSW) in Japan, based on the five microsatellites loci.

originating in the founder populations introduced to shrines moved to the neighbouring tea plantations in Kyoto. The number of invasion events of TSW in Japan could not be estimated because the single haplotype (B1) was mostly fixed in the putative source area i.e., China.

Another phylogenetic group (haplogroup B3) was only detected on Theaceae plants in Japanese quarantine. Given its close phylogenetic relationships with haplotype B1 and B2, B3 might become a third invasive phylogenetic group in tea plantations in both China and Japan. To estimate the risk of invasion it will be necessary to study the feeding and oviposition behaviour of haplogroup B3 on host leaves and to continuously monitor populations for possible outbreaks in tea plantations.

Genetic variation of *E. smithi* and its relationships with the two species of spiny whiteflies

Although PCR amplification using the universal primers C1-J-2195 and L2-N-3014 has been inefficient for some species, it successfully amplified all *E. smithi* mtCOI in this study. Two phylogenetic groups (haplotype I and haplogroup II) were found in Japan and, in most cases, haplotype I and haplogroup II were associated with OSW and TSW, respectively. These results support the hypothesis that *E. smithi* parasitizing TSW did not originate from an existing population parasitizing OSW in Japan, instead arriving in this country following the invasion of TSW. In rare cases, haplotype I was parasitizing TSW in the populations SHZ5 and FKO2. This was probably due to the migration of haplotype I from an orange orchard to a tea plantation where the ecological niche was empty because haplogroup II had not immigrated. Because 27.9% of the SHZ5 population was parasitized by wasp pupae (Uesugi, unpublished data), *E. smithi* haplotype I is a potential biocontrol agent towards field populations of TSW and OSW.

Microsatellites usually reveal more recent demographic history, such as bottlenecks or expansion, than mtCOI (Selkoe & Toonen, 2006). The genetic structure of *E. smithi* detected by the microsatellites was largely affected by demographic events after their introduction in Japan. A higher level of genetic variation in haplotype I was detected in the populations from the main islands when compared with those of the Nansei islands in Japan, and populations from the main islands were genetically similar to each other. Presumably, *E. smithi* populations in the main islands originated from the populations introduced during the biological control programs initiated in 1925 by the Japanese government. In such programs, the release of as much genetic variation as possible into the new region is often encouraged to enhance the success rate (Simmonds, 1963; Phillips *et al*., 2008). Therefore, the main island populations have preserved a high level of genetic variation and similar allelic frequencies throughout the years. Contrarily, the introduction of *E. smithi* in the Nainsei islands, which appears unintentional or performed on a private basis because there is no record concerning an organized introduction program, resulted in a genetic bottleneck and severely decreased genetic diversity in this region. Similarly, haplogroup II might have been unintentionally introduced to tea plantations in distant regions, along with the human-aided movement of TSW in Japan, experiencing severe genetic bottlenecks that resulted in a low level of genetic diversity and a high level of genetic differentiation between wasp populations.

In parasitoid wasps, the genetic differentiation associated with different host species was often reported as host specialization. Kankare *et al*. (2005) found two genetic groups of microsatellites in *Cotesia melitaearum* (Wilkinson), a parasitoid wasp of checkerspot butterflies, which were associated with two different host species (*Melitaea cinxia* (L.) and *Euphydryas aurinia* (Rottemburg)). They concluded that this data was one of the evidence of coevolution between the

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Table 4. Mutation-scaled effective population sizes (Θ; $\Theta = 4N_e\mu$) and numbers of effective migrants per generation (N_em; $N_em = \Theta M/4$) in the ten *Encarsia smithi* populations based on five microsatellites loci. See table 1 for population codes.

Population (*i*)	Θ	N_em									
		SHZ1-> *i*	SHZ2-> *i*	EHI-> *i*	FKO1-> *i*	AMA1-> *i*	OKI-> *i*	IRI1-> *i*	GIF-> *i*	MIE-> *i*	KYO2-> *i*
SHZ1	0.545	–	0.391	0.587	0.386	1.67	0.304	0.717	0.630	1.04	1.09
SHZ2	0.739	0.389	–	0.410	0.573	1.23	0.471	0.677	1.47	1.21	1.35
EHI	0.741	0.0494	0.0330	–	0.198	0.567	0.247	0.890	0.561	1.01	0.973
FKO1	0.591	0.315	0.727	0.0373	–	0.949	0.0746	0.243	0.149	0.131	0.000
AMA1	0.678	0.593	0.593	0.319	0.253	–	0.0769	0.417	0.0879	0.220	0.000
OKI	0.477	0.0997	0.0249	0.0872	0.411	0.000	–	0.336	0.150	0.287	0.000
IRI1	0.525	0.0966	0.0552	0.145	0.000	0.000	0.0138	–	0.000	0.148	0.000
GIF	0.573	0.373	0.440	0.230	0.0765	0.0382	0.124	0.153	–	0.0191	0.000
MIE	0.602	0.742	0.753	0.306	0.0118	0.0353	0.000	0.188	0.200	–	0.593
KYO2	0.582	0.370	1.29	1.86	0.507	0.117	0.897	0.137	0.585	0.1559	–

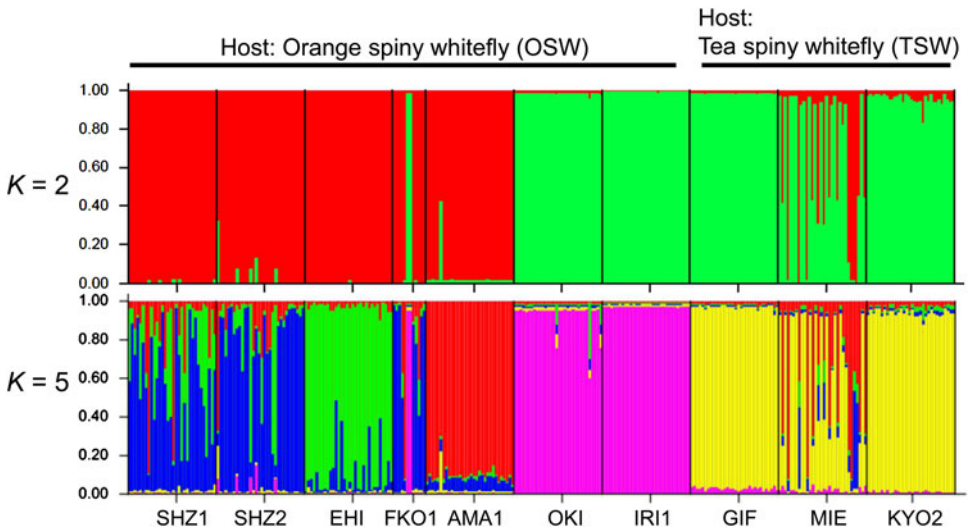

Fig. 7. Estimated population structure of *Encarsia smithi* parasitizing the orange spiny whitefly (OSW) and the tea spiny whitefly (TSW) in Japan, based on the five microsatellites loci and using the program Structure 2.3 ($K = 2$ and $K = 5$). Thin vertical lines, partitioned into K coloured segments that represent the estimated membership clusters, represent individuals. Black lines separate the different populations.

butterflies and the parasitoid wasps because of the reciprocal ecological dynamics occurring in this host-parasitoid system. Stireman *et al*. (2006) also found two genetic lineages in the mtCOI of the parasitoid wasp *Platygaster variabilis* Fouts, 1924, which were associated with two genetically differentiated lineages of the goldenrod gall makers *Rhopalomyia solidaginis* (Loew) specializing in two species of sympatric host plants. They concluded that the two lineages of the parasitoid wasps had diversified sympatrically in association with the diversification of their hosts, based on the distribution and patterns of divergence showed by hosts and gall makers. Such host specialization of parasitoid wasps is also well-known in many species of the subfamily Aphidiinae (Starý, 1981). However, the two phylogenetic groups of *E. smithi* (haplotype I and haplogroup II) did not result from their specialization in OSW and TSW, respectively, as this wasp can parasitize different species of *Aleurocanthus* whiteflies (Heraty *et al*., 2007*b*; Dubey & Ko, 2012). In addition, haplotype I parasitized TSW field populations (table 2) and haplogroup II could sexually reproduce by parasitism on OSW in the laboratory (Uesugi *et al*., unpublished data). Hence, the host associations observed in the present study was most likely shaped by the recent introductions and expansion of haplogroup II along with human-aided movement of TSW in Japan and, in a near future, the two phylogenetic groups of wasps might have overlapping host species in Japan.

Implications for the control of TSW

Genetics should play a larger role in the development of invasive species management and control policies and in the defence against controlling agents (Allendorf & Lundquist, 2003). Although the economic impact of OSW and TSW infections is becoming a serious worldwide problem, and their natural enemy *E. smithi* has proven to be an excellent agent to control them in field studies, little genetic information exists regarding their region of origin and phylogenetic diversity. Although our genetic analysis was limited to China and Japan, it has important implications for the control of these two invasive whitefly pests as follows.

First, a better understanding of the phylogenetic relationships within the two whitefly species will allow estimating

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the risk of invasion by other phylogenetic groups. For example, tobacco whitefly *Bemisia tabaci* (Gennadius) has several biotypes that differ in their invasive potential. Biotype Q has a tolerance of extreme temperatures (Bonato *et al*., 2007) and insecticides (Horowitz *et al*., 2005; Tsagkarakou *et al*., 2007). Therefore, following the colonization by biotype B, the immigration of biotype Q caused additional problems for agriculture (Guirao *et al*., 1997; Palumbo *et al*., 2001; Nauen *et al*., 2002). Determining whether the same problem can also occur in the two spiny whiteflies is necessary, and hence further studies regarding the ecological characteristics of the several phylogenetic groups, such as feeding behaviour and reproductive isolation, is required. In addition, colonization from multiple sources might mitigate the loss of genetic diversity in an invaded area and may result in novel selection challenges being encountered in the recently invaded area (Facon *et al*., 2006; Dlugosch & Parker, 2008; Verhoeven *et al*., 2011); therefore, preventing additional invasions from occurring is desirable (Frankham, 2005).

Second, we should investigate the effective use of the two phylogenetic groups of the parasitoid wasp *E. smithi* for the biological control of TSW in Japan. Because the genetic diversity of haplogroup II was low in Japan, *E. smithi*'s performance against TSW could be improved by releasing individuals obtained from the native range that would maximize its genetic diversity (Phillips *et al*., 2008). It is therefore necessary to study if the wasp's genetic diversity is linked to the ability to suppress pest populations and adapt to changes in host population dynamics (Hopper *et al*., 1993). In addition, we should consider any undesirable side effects on the ecosystem likely to be caused by haplogroup II. The wasp was not detected in parasitoid wasp surveys performed in tea plantations before TSW was introduced in Japan (Takagi, 1974). Therefore, haplogroup II was probably introduced to Japan following the invasion of TSW. For example, a negative effect on haplotype I can be caused by competition and hybridization, or a negative effect on minor species of spiny whiteflies such as *Aleurocanthus cinnamomi* Takahashi can occur by parasitism. The additional release of haplotype I parasitizing OSW, which preserves a high level of genetic variation, might also be effective for the control of TSW, as parasitism by both haplotype I and haplogroup II could have a synergistic effect and better suppress TSW populations in Japan.

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