

Distribution and population structure of two phylogroups of the parasitoid *Encarsia smithi* (Hymenoptera: Aphelinidae) in tea fields infested with the invasive camellia spiny whitefly *Aleurocanthus camelliae* (Hemiptera: Aleyrodidae) in Shizuoka Prefecture, Japan

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## Applied Entomology and Zoology

### Distribution and population structure of two phylogroups of the parasitoid *Encarsia smithi* (Hymenoptera: Aphelinidae) in tea fields infested with the invasive camellia spiny whitefly *Aleurocanthus camelliae* (Hemiptera: Aleyrodidae) in Shizuoka Prefecture, Japan

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Abstract:	<p>A recent study revealed that two phylogenetic groups of the parasitoid <i>Encarsia smithi</i> (Hymenoptera: Aphelinidae) can attack the camellia spiny whitefly <i>Aleurocanthus camelliae</i> (Hemiptera: Aleyrodidae), an invasive pest of Japanese tea fields. Type I was introduced in 1925 from China to Japanese citrus orchards to control the citrus spiny whitefly <i>A. spiniferus</i>, but it has also recently appeared in several tea fields. Type II, presumably introduced accidentally, was also found in many tea fields. However, little is known about distribution and their relative importance as a biocontrol agent in tea fields. To investigate these aspects, we developed specific PCR for the two groups using a variation in their nuclear ribosomal DNA's ITS region. We then surveyed their distribution in 23 tea fields in Shizuoka Prefecture, Japan, from 2013 to 2015 using this specific PCR. We found that both types were distributed, sometimes coexisting, in many tea fields during 2013 - 2015, although the population structure of these types varied with the field, year and season. These results suggest that <i>A. camelliae</i> can be controlled unintentionally by accidentally-introduced exotic natural enemies (Type II) and/or Type I species originally introduced to control other invasive pests such as <i>A. spiniferus</i>.</p>
Response to Reviewers:	<p>Dear Dr. Kainoh,</p> <p>We are most grateful to you and the reviewers for your helpful comments on our revised manuscript. We accepted all the advice and have carried out the corrections suggested. We trust that the revised version of our paper is now suitable for publication in <i>Applied Entomology and Zoology</i>.</p>

Gratefully,  
Kaori Yara

Reviewers' comments:

I have only a few comments. I also used the "track change in Word" to indicate the style corrections. Please download the file.

Response: We have corrected the style of our manuscript according to the "track change in Word".

Table 3:

Numbers preceding name of city or town correspond to those in Figure 2.

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Response: We have corrected the revised Table 3.

We hope our responses are satisfactory. We look forward to your consideration.

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Kaori Yara

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1     **1     Distribution and population structure of two phylogroups of the parasitoid**  
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3     **2     *Encarsia smithi* (Hymenoptera: Aphelinidae) in tea fields infested with the invasive**  
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5     **3     camellia spiny whitefly *Aleurocanthus camelliae* (Hemiptera: Aleyrodidae) in**  
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7     **4     Shizuoka Prefecture, Japan**  
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13     **6     Kaori Yara<sup>1†</sup> · Ryuji Uesugi<sup>1§</sup> · Takeshi Shimoda<sup>2</sup> · Yasushi Sato<sup>1</sup>**  
14  
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1    21    **Abstract**

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3    22    A recent study revealed that two phylogenetic groups of the parasitoid *Encarsia smithi*  
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6    23    (Hymenoptera: Aphelinidae) can attack the camellia spiny whitefly *Aleurocanthus*  
7  
8    24    *camelliae* (Hemiptera: Aleyrodidae), an invasive pest of Japanese tea fields. Type I was  
9  
10    25    introduced in 1925 from China to Japanese citrus orchards to control the citrus spiny  
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12    26    whitefly *A. spiniferus*, but it has also recently appeared in several tea fields. Type II,  
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14    27    presumably introduced accidentally, was also found in many tea fields. However, little  
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16    28    is known about distribution and their relative importance as a biocontrol agent in tea  
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18    29    fields. To investigate these aspects, we developed specific PCR for the two groups using  
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20    30    a variation in their nuclear ribosomal DNA's ITS region. We then surveyed their  
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22    31    distribution in 23 tea fields in Shizuoka Prefecture, Japan, from 2013 to 2015 using this  
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24    32    specific PCR. We found that both types were distributed, sometimes coexisting, in many  
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26    33    tea fields during 2013–2015, although the population structure of these types varied  
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28    34    with the field, year and season. These results suggest that *A. camelliae* can be controlled  
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30    35    unintentionally by accidentally-introduced exotic natural enemies (Type II) and/or Type  
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32    36    I species originally introduced to control other invasive pests such as *A. spiniferus*.

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41    38    **Keywords**

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43    39    *Aleurocanthus camelliae* · *Encarsia smithi* · parasitoid wasp · phylogenetic group ·  
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45    40    specific PCR

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1     **42 Introduction**

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3     43 Biological control is an effective means of controlling agricultural pests (Cock et al.  
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5     44 2016; van Lenteren 2012; Van Driesche et al. 2010). Exotic natural enemies are often  
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7     45 employed to control invasive pests (Hajek et al. 2016). Many studies have demonstrated  
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9     46 successful control of invasive pests by intentionally-introduced biocontrol agents  
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11     47 (Bellows 2001; Cock et al. 2016). However, fewer studies have documented the  
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13     48 unintentional suppression of invasive pests by accidentally-introduced natural enemies  
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15     49 or species already introduced to control other invasive pests (Hajek et al. 2016; Kenis et  
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17     50 al. 2017). This paper focuses on the unexpected impact of exotic enemies on invasive  
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19     51 pests observed in Japanese tea fields.

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25     52         *Encarsia smithi* (Silvestri) (Hymenoptera: Aphelinidae) is a well-known parasitoid  
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27     53 of the citrus spiny whitefly *Aleurocanthus spiniferus* (Quaintance) and *A. woglumi*  
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29     54 Ashby (Hemiptera: Aleyrodidae), both citrus pest whiteflies, in many countries (Nguyen  
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31     55 and Sailer 1987). In Japan, *E. smithi* was first introduced from southern China to  
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33     56 Nagasaki Prefecture in 1925 to control an *A. spiniferus* invasion in a citrus orchard  
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35     57 (Kuwana 1934). The parasitoid was then mass-released in other areas from 1961 to  
36  
37     58 1998 as part of a government project. These intermittent releases resulted in a drastic  
38  
39     59 decrease in *A. spiniferus* populations in most areas, except for the occasional occurrence  
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41     60 in limited regions (Ohgushi 1969). This classical biological control is one of the best-  
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43     61 known and most successful ones in Japan (van den Berg and Greenland 1997).

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45     62         Interestingly, *E. smithi* has recently reappeared in many tea fields as a significant  
46  
47     63 natural enemy of the invasive camellia spiny whitefly *A. camelliae* Kanmiya and Kasai,  
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49     64 the occurrence of which was first reported in Japan in 2004 (Yamashita et al. 2016).  
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51     65 Uesugi et al. (2016a) reported, using mtDNA COI and microsatellite variability, that the

1 66 *E. smithi* samples collected from nine tea fields comprised two phylogenetic groups:  
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3 67 Type I was observed in two fields (in Shizuoka and Fukuoka Prefectures), while Type II  
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5 68 was identified in another seven fields (in Shizuoka, Gifu, Mie, Shiga, Kyoto, Nara and  
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7 69 Hyogo Prefectures). They also reported that all the *E. smithi* individuals collected from  
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9 70 ten citrus orchards were Type I. It therefore appears that Type I is derived from the  
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11 71 populations released for controlling *A. spiniferus*, while Type II might have been  
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13 72 unintentionally introduced to tea plantations alongside the invasion of *A. camelliae*  
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15 73 (Uesugi et al. 2016a).  
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21 74 Although each type of *E. smithi* can parasitize *A. camelliae* in tea fields, their  
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23 75 relative importance as biological control agents remains to be evaluated. Whether Type I  
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25 76 frequently predominates over Type II in tea fields is particularly open to question. The  
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27 77 ecological characteristics of each type have also not been closely studied. For example,  
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29 78 it is unclear whether they tend to be distributed separately in different tea fields or  
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31 79 coexist in the same tea fields; the results of Uesugi et al. (2016a) may suggest the  
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33 80 former possibility. Further, their intraspecific interaction (e.g., competition or  
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35 81 hybridization) has never been examined. To clarify these matters, we need to develop a  
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37 82 new molecular method of identifying their hybrids as well as each group.  
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42 83 In this study, we first developed PCR specific to the two phylogroups by using a  
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44 84 variation in the ITS region of their nuclear ribosomal DNA. Thereafter, by using this  
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46 85 specific PCR, we surveyed the population structures of the types in tea fields in  
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48 86 Shizuoka Prefecture over three years. The relative importance of these types as a natural  
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50 87 enemy and their complex ecological interactions in tea fields and citrus plants were  
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52 88 investigated.  
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1 90 **Materials and methods**

2  
3 91 **Developing specific PCR**

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6 92 *Encarsia smithi*, listed in Table 1, were used to sequence the internal transcribed spacer  
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8 93 (ITS) of the nuclear ribosomal DNA (nrDNA) region and to a develop specific PCR to  
9  
10 94 distinguish the two phylogroups. They were already part of the samples taken by Uesugi  
11  
12 95 et al. (2016a) and their phylogroups had been already been identified. DNA was  
13  
14 96 extracted from these individuals with a DNeasy Blood & Tissue Kit (Qiagen) in  
15  
16 97 accordance with the manufacturer's instructions. The DNA was eluted with 50 µl of  
17  
18 98 Buffer AE from the kit. The DNA sequences of the ITS region were determined  
19  
20 99 according to Schmidt et al. (2006) with some modifications. To amplify the ITS region,  
21  
22 100 two primers, TW81 5'-GTTTCCGTAGGTGAACCTGC-3' and Aed5.8R 5'-  
23  
24 101 GAGAACAGCAGGAACACAGAAC-3' (Brust et al. 1998) were used. PCR was  
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26 102 carried out in 25-µl reaction mixtures containing 0.2 mM each of dNTP, 0.2 µM of each  
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28 103 primer, 1.0 µl template DNA, 0.65 U of *TaKaRa Ex Taq* (Takara Bio Inc.), and 1× *Ex*  
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30 104 *Taq* buffer (2.0 mM Mg<sup>2+</sup> concentration). The cycling conditions were as follows: 94 °C  
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32 105 for 5 min, 40 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1.5 min, and 72 °C  
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34 106 for 5 min in a GeneAmp PCR System 9700 (Perkin-Elmer). PCR products were purified  
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36 107 using ExoSAP-IT (Affymetrix), and both strands were directly sequenced using the  
37  
38 108 same primers as in the PCR and a BigDye Terminator v3.1 Cycle Sequencing Kit  
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40 109 (Applied Biosystems) in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems),  
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42 110 according to the manufacturer's instructions.

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44 111 From the ITS sequence data obtained (DDBJ/EMBL/GenBank Acc# LC326520,  
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46 112 LC326521), we designed four specific primers that included specific InDel regions:  
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48 113 ITS1-EsI-F, ITS2-EsI-R, ITS1-EsII-F, and ITS2-EsII-R (Table 2). Specific PCR using  
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1 114 the designed primers was carried out under almost the same conditions as described  
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3 115 above, but the cycling conditions were as follows: 96 °C for 1 min, 40 cycles at 94 °C  
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5 116 for 1 min, 57 °C for 1 min, 72 °C for 1.5 min, and 72 °C for 7 min on a MyCycler (Bio-  
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8 117 Rad Laboratories, Inc.). The PCR products were electrophoresed in 2% agarose gel in  
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10 118 1×TAE buffer at 100 V for 30 min and visualized by staining with GelRed Nucleic Acid  
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12 119 Gel Stain (Biotium).

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15 120 The designed primers' specificity was validated using the same specimens that were  
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17 121 used to determine the ITS sequences (Table 1). Their usability in this study was  
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19 122 confirmed by using specimens that had been taken from the same population in Table 1  
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21 123 but with their DNA extracted with PrepMan Ultra Reagent (Applied Biosystems) as  
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23 124 described below.

#### 24 25 125 **Sample collection**

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28 126 *Encarsia smithi* were collected from 24 tea fields in Shizuoka Prefecture, Japan, from  
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30 127 2013 to 2015 (Fig. 1, Table 3). Leaves of the tea plant *Camellia sinensis* infested with  
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32 128 the host, camellia spiny whitefly *Aleurocanthus camelliae* nymphs, were collected from  
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34 129 April to August of each year (Table 3), placed in plastic boxes, and kept in an  
35  
36 130 incubation room (23 °C, 15L 9D). Adult *E. smithi* that emerged from the hosts were  
37  
38 131 collected and stored in 99.5 % ethanol at 4 °C until genetic analysis.

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40 132 One *E. smithi* adult that emerged from another host, the citrus spiny whitefly *A.*  
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42 133 *spiniferus* infesting a citrus leaf, was also used (Table 3). It was obtained in a similar  
43  
44 134 manner to that described above: a citrus leaf infested with *A. spiniferus* was collected in  
45  
46 135 August of 2014. The citrus tree from which the leaf was collected was surrounded by  
47  
48 136 tea fields.

#### 49 50 137 **Identification by type-specific PCR**

1 138 DNA was extracted from single, whole specimens using PrepMan Ultra Reagent  
2  
3 139 (Applied Biosystems) according to the manufacturer's instructions, not homogenizing  
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5 140 but instead vortexing the body. 30 µl of reagent was used per individual. For each  
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7 141 individual, two kinds of specific PCR, using two primers (ITS1-EsI-F and ITS2-EsI-R)  
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9 142 specific to Type I, and using the other two primers (ITS1-EsII-F and ITS2-EsII-R)  
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11 143 specific to Type II (Table 2), were carried out. The PCR and electrophoretic conditions  
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13 144 were the same as described above.  
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18 145 The specimens were identified as Type I when they produced the expected amplicon  
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20 146 (ca. 993 bp band) by PCR specific to Type I only; the specimens were identified as Type  
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22 147 II when they produced the expected amplicon (ca. 890 bp band) by PCR specific to  
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24 148 Type II only; and the specimens were identified as hybrids when they produced the  
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26 149 expected amplicons by both the specific PCRs.  
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30 150 The variation in frequency of genetic types of *E. smithi* for different dates of  
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32 151 collection was tested using the generalized linear model (GLM) with a binomial  
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34 152 distribution at each sampling location using R (version 3.4.0) statistical software. The  
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36 153 likelihood ratio test was performed using "car" package versions 2.1 - 6 in R version  
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38 154 3.4.0. In the model, data from hybrid genotypes was not used.  
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## 43 156 **Results**

### 44 157 **Specific PCR**

45 158 We obtained approximately 1000-bp sequences containing 18S rDNA, ITS1, 5.8S  
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47 159 rDNA, and ITS2 region (Acc# LC326520, LC326521). Sufficient sequence data was  
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49 160 obtained to design specific primers to two phylogroups by inspecting electropherograms  
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51 161 and by distinguishing between intra- and inter-phylogroup variation. The specific  
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1 162 primers we designed produced the expected amplicons in each of two specific PCRs  
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3 163 (Fig. 2). When two phylogroups' DNAs were mixed at a ratio of 1 : 1 and used as  
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5 164 template DNA, both amplicons were produced (Fig. 2). Consistent results were obtained  
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7  
8 165 when we used DNA templates prepared using PrepMan Ultra Reagent. Specific PCR  
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10 166 was therefore conducted to determine the phylogroup for each *E. smithi* and the hybrid  
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12  
13 167 between them.  
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15 168

### 169 Occurrence of the two phylogroups and their hybrid

170 The specific PCR revealed two types of *E. smithi* and their F<sub>1</sub> hybrid in this study. The  
171 GLM suggests that the frequency of the two types changed significantly with different  
172 collection dates in 10 locations (Fujinomiya 3-4, Shizuoka 2-3, Fujieda, Shimada 1-2,  
173 Hamamatsu 1, 3-4;  $p < 0.05$ , likelihood ratio test: Fig. 3). However, no general patterns  
174 in population structure changes were observed in these fields. For example, all  
175 individuals collected from Shimada 1 in 2013 and 2015 were identified as Type I and  
176 Type II, respectively. Type I predominated over Type II at Shizuoka 3 and Fujieda in  
177 2013, while the opposite pattern was observed in each of these fields in 2015. Type II  
178 predominated over Type I at Hamamatsu 1 and 4 in 2013, and all individuals from  
179 Hamamatsu 4 in 2014 were Type II; but Type I predominated over Type II in each field  
180 in 2015. At Fujinomiya 3 and Hamamatsu 3, Type II predominated over Type I in 2013,  
181 while all specimens from this field in 2015 were identified as Type I.

182 The GLM suggests that frequency of the two types did not change significantly  
183 among different collection dates in the other nine locations (Numazu 1 and 2,  
184 Fujinomiya 1 and 2, Shizuoka 1 and 4, Makinohara, Kawanehoncho and Hamamatsu 2;  
185  $p > 0.05$ , likelihood ratio test: Fig. 3). For example, all individuals collected from



1 186 Fujinomiya 1 and 2 and Shizuoka in 2013 and 2015 were identified as Type I. This was  
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3 187 also the case for parasitoids collected from Makinohara in 2013 and the spring of 2014.  
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5 188 The dominant type in Numazu 2 was Type I in 2013 and 2015. On the other hand, Type  
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8 189 II predominated over Type I at Kawanehoncho in 2013, 2014 (summer) and 2015.  
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10 190 In this study, a total of 491 parasitoids were collected in the tea fields over the three  
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13 191 years, of which 277 and 208 individuals (56.4 % and 42.4 %) were respectively  
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15 192 identified as Type I and Type II. Only six individuals that remained (1.2 %) were  
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18 193 identified as their F<sub>1</sub> hybrids. Type I was observed in all the 23 tea fields investigated in  
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20 194 2013 (100 %), while Type II was observed in 12 fields in the same year (50.2 %) (Fig.  
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22 195 3). We also found Type I in 14 fields (77.8 %), and Type II in 10 fields (55.6 %), out of  
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25 196 18 fields investigated in 2015.

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27 197 Although we did search for *E. smithi* on citrus leaves infested with *A. spiniferus*,  
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29 198 only one parasitoid was eventually collected from this plant-host combination.  
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31 199 Interestingly, this individual was identified as Type II.  
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## 36 201 Discussion

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38 202 In Shizuoka Prefecture, the invasive pest *A. camelliae* was first found in a tea field in  
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40 203 Kikugawa (Kurasawa) in October 2010; the species then rapidly expanded its  
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43 204 distribution throughout the prefecture in 2011–2012 (Ozawa et al. 2015). Ozawa et al.  
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45 205 (2015) also found *E. smithi* in most of the tea fields they investigated (105 of 121 fields)  
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48 206 located in various regions in the prefecture from December 2012 to March 2013, such  
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51 207 as Numazu, Fujinomiya, Shizuoka, Shimada, Makinohara and Hamamatsu. We also  
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54 208 collected *E. smithi* from the same regions or local areas and then found that 1) both  
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57 209 types were distributed, sometimes coexisting, in many tea fields in Shizuoka Prefecture  
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1 210 in 2013–2015; and 2) one type sometimes predominated over the other in these fields.  
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3 211 There are a few field studies suggesting that parasitism by *E. smithi* (type unknown)  
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5 212 affected the density of *A. camelliae* in tea fields in Shizuoka Prefecture (Ozawa et al.  
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7  
8 213 2015; Uesugi et al. 2016b). From these results and previous findings, we can say that  
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10 214 each type of *E. smithi* played a role in controlling *A. camelliae* in the tea fields  
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12 215 investigated. Further, to the best of our knowledge, this is the first report to show that  
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14 216 populations of an invasive pest (*A. camelliae*) can be controlled unintentionally by  
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16 217 accidentally-introduced exotic enemies (Type II) and/or exotic but already established  
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18 218 species used as control agents against other invasive pests (Type I and *A. spiniferus*,  
19  
20 219 respectively).

25 220 Interestingly, Type I was already present in all of the 23 tea fields investigated up  
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27 221 until 2013, whereas Type II had been distributed in half of these fields until that year  
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29 222 (Fig. 3). A plausible explanation for the more frequent observation of Type I is that they  
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31 223 were endemic to the regions investigated. In Shizuoka Prefecture, both citrus and tea are  
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33 224 intensively cultivated, sometimes close to each other, in the same regions. It is likely  
34  
35 225 that Type I parasitoids that had inhabited citrus trees with *A. spiniferus* had dispersed to  
36  
37 226 neighboring tea fields to exploit *A. camelliae* as a new host. After Type I had established  
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39 227 populations in the tea fields, they might have rapidly dispersed to other tea fields in the  
40  
41 228 same local areas to exploit the abundant available hosts. It appears that *E. smithi* (type  
42  
43 229 unknown) in tea fields has rapidly expanded its distribution via frequent aerial dispersal  
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45 230 between tea fields (Uesugi et al. 2016b). Further studies are needed to clarify the  
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47 231 expansion of distribution of each type and its underlying mechanisms, as well as the  
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49 232 consequences of biological pest control applied using these types in tea fields.  
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57 233 We found that the two types of parasitoids sometimes coexisted in the same tea  
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1 234 fields (Fig. 3). In our previous study (Uesugi et al. 2016a), they were observed  
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3 235 allopatrically in tea fields. In this study, in contrast, both types were sympatrically found  
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5 236 in 12 of 23 tea fields in 2013, and 6 of 18 fields in 2015 (Fig. 3). This prompts the  
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7 237 interesting question as to whether there was an intense competitive interaction between  
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9 238 the two types of parasitoids, causing the displacement of one type by the other. One  
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11 239 example of this is the displacement of *Torymus beneficus* (Hymenoptera: Torymidae) by  
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13 240 *T. sinensis*, a simultaneously indigenous and introduced parasitoid of the chestnut gall  
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15 241 wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), in Japanese chestnut  
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17 242 plantations (Yara et al. 2007, 2010). In this study, however, no fiercely competitive  
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19 243 interaction was found between them, and no general pattern of change in their  
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21 244 population structure. The only evidence of any interaction was hybridization, although it  
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23 245 was detected only rarely (Fig. 3). The influence of hybridization and other possible  
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25 246 interactions between the two parasitoids on the outcome of the biological control  
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27 247 remains to be evaluated in further studies.

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29 248 This is the first report to show that Type II can inhabit the vegetation surrounding  
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31 249 tea fields. In our previous study, Type II was collected from tea plants only, whereas  
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33 250 Type I was collected from citrus plants, (e.g., *Citrus unshiu*) as well as from tea plants  
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35 251 (Uesugi et al. 2016a). Because *A. spiniferus* is generally distributed at low density in  
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37 252 citrus plants, we were able to obtain only one parasitoid, identified as Type II, from a  
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39 253 citrus tree infested with *A. spiniferus* beside a tea field. We conclude that each type of *E.*  
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41 254 *smithi* can exploit both tea plants and the surrounding vegetation infested with different  
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43 255 host species.

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45 256 Our findings in this study suggest complex interactions among the parasitoids,  
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47 257 whiteflies and host plants. Previous studies have focused on simple interactions between  
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1 258 *E. smithi* (mainly Type II) and *A. camelliae* as a pest of tea (Ozawa et al. 2015; Uesugi  
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3 259 et al. 2016b) or between *E. smithi* (Type I) and *A. spiniferus* as a citrus pest (Kawamura  
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5 260 1976) (Fig. 4a). Other than this, our study makes clear the importance of Type I,  
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7 261 dispersing from surrounding vegetation, as a biocontrol against *A. camelliae* (Fig. 4b).  
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9 262 Our results also suggest the possibility of intraspecific interactions between the two  
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11 263 types of parasitoids in tea fields and the surrounding vegetation (e.g., citrus), although  
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13 264 their influence on the biological control of the two whiteflies remains to be evaluated.  
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15 265 Further field and laboratory studies should aim to explore these complicated interactions  
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17 266 in tea fields and the surrounding vegetation and to clarify the ecological characteristics  
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19 267 of these parasitoids. In a previous study, we found that Type I and Type II have different  
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21 268 ecological characteristics in terms of number of offspring and emergence patterns, when  
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23 269 rearing them using citrus seedlings infested with *A. spiniferus* (Yara et al. 2017). This  
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25 270 pattern may be also seen in their relationship with *A. camelliae*, although we need a  
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27 271 rearing method for the parasitoids using this host.  
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39 274 their extremely helpful comments.  
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**345 Figure legends**

**346 Fig. 1** A map of the Japanese Archipelago. Shizuoka Prefecture, the study region in  
**347** this article, is circled.

**348**  
**349 Fig. 2** PCR amplification with the specific primer pairs for each phylogroup of  
**350** *Encarsia smithi*. a: primers specific to Type I used; b: primers specific to Type  
**351** II used. Lanes 1 - 2: Type I; 3 - 4: Type II; 5-6: DNAs of Type I and II were  
**352** mixed at a ratio of 1 : 1 and used as template DNA. M: 100 bp ladder marker.

**353**  
**354 Fig. 3** Population structure of the two phylogroups of *Encarsia smithi* and their  
**355** hybrids in tea fields in Shizuoka Prefecture in 2013–2015. The numbers in the  
**356** small circles on the map, which represent the sampling sites, correspond to  
**357** those tabulated in Table 3. The numbers in the pie charts indicate the numbers  
**358** of parasitoids (Type I, Type II and their hybrids) found at each sampling site.  
**359** Asterisks indicate significant differences among different years of collection ( $p$   
**360**  $< 0.05$ , likelihood ratio test).

**361**  
**362 Fig. 4** A schematic illustration of the observed interactions among parasitoids, host  
**363** whiteflies, and host plants in (a) previous studies and (b) the present study. Thick  
**364** arrows show frequently observed interactions or events, and thin arrows show  
**365** less frequently observed interactions or events.

Fig.1

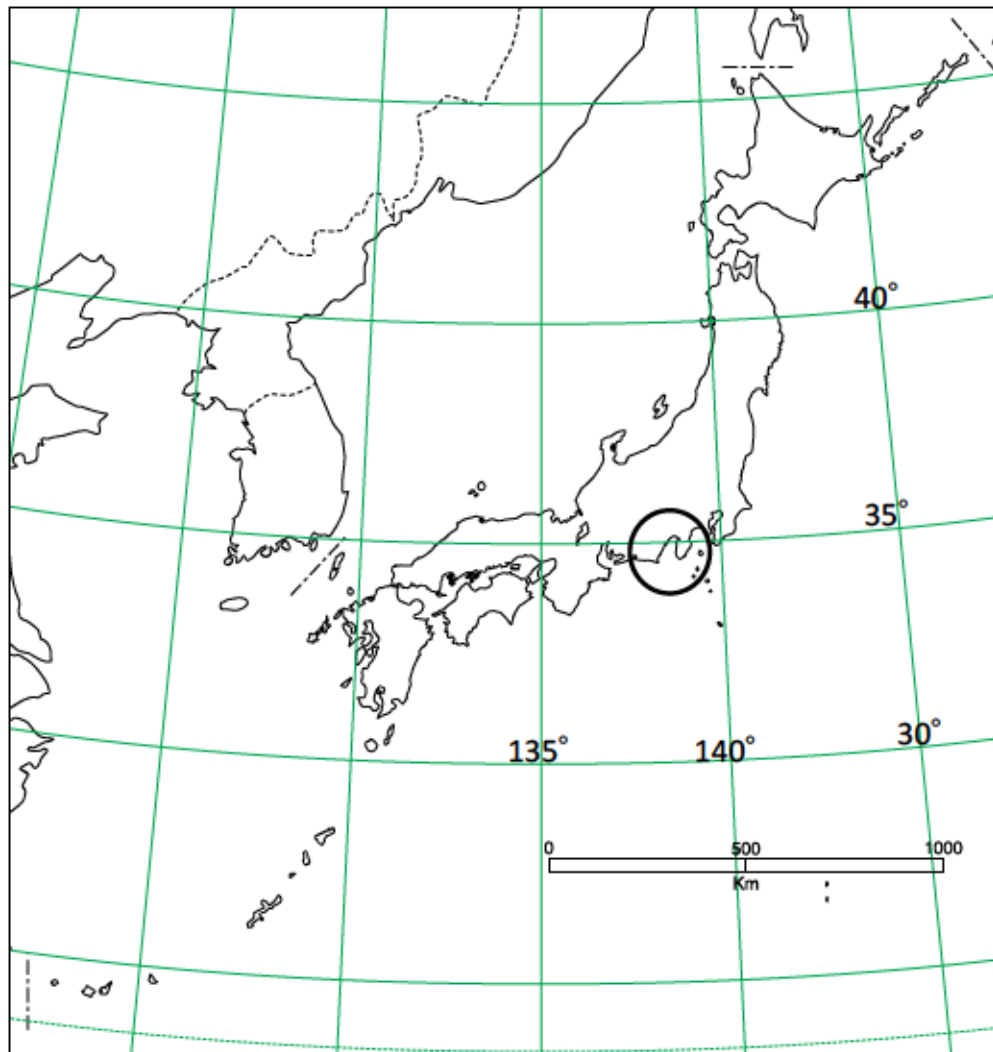


Fig. 2

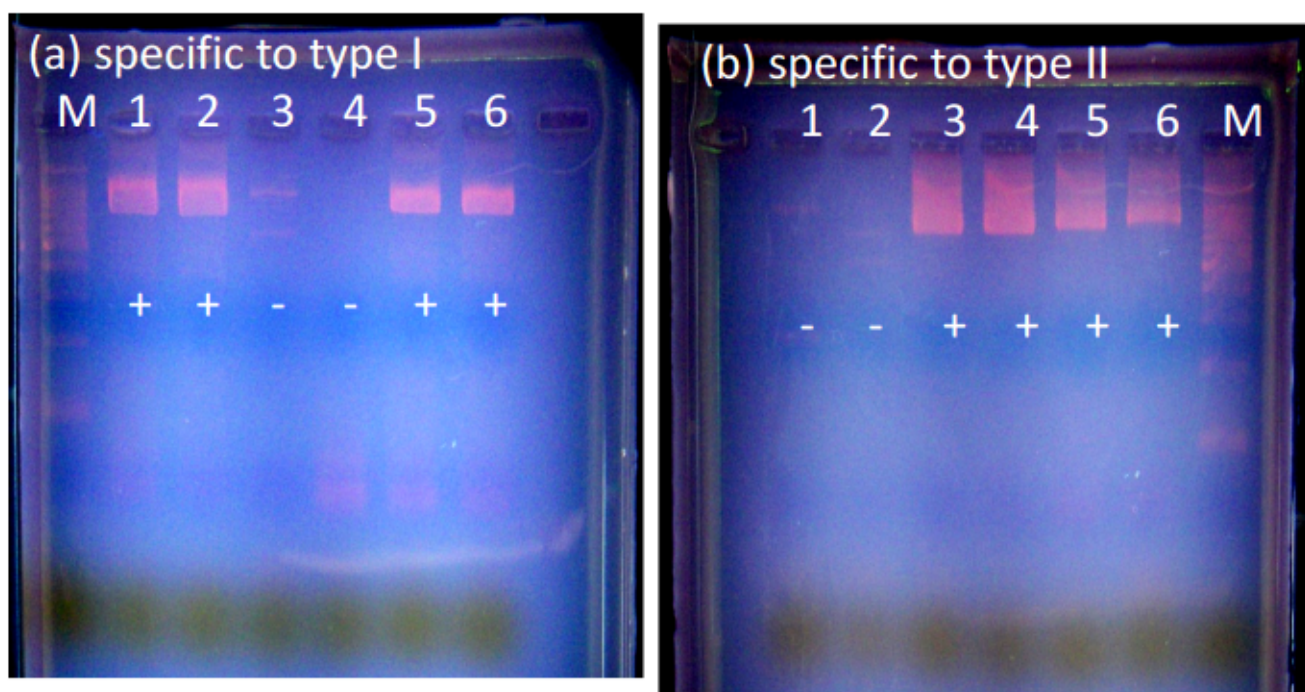


Figure 3

Fig.3

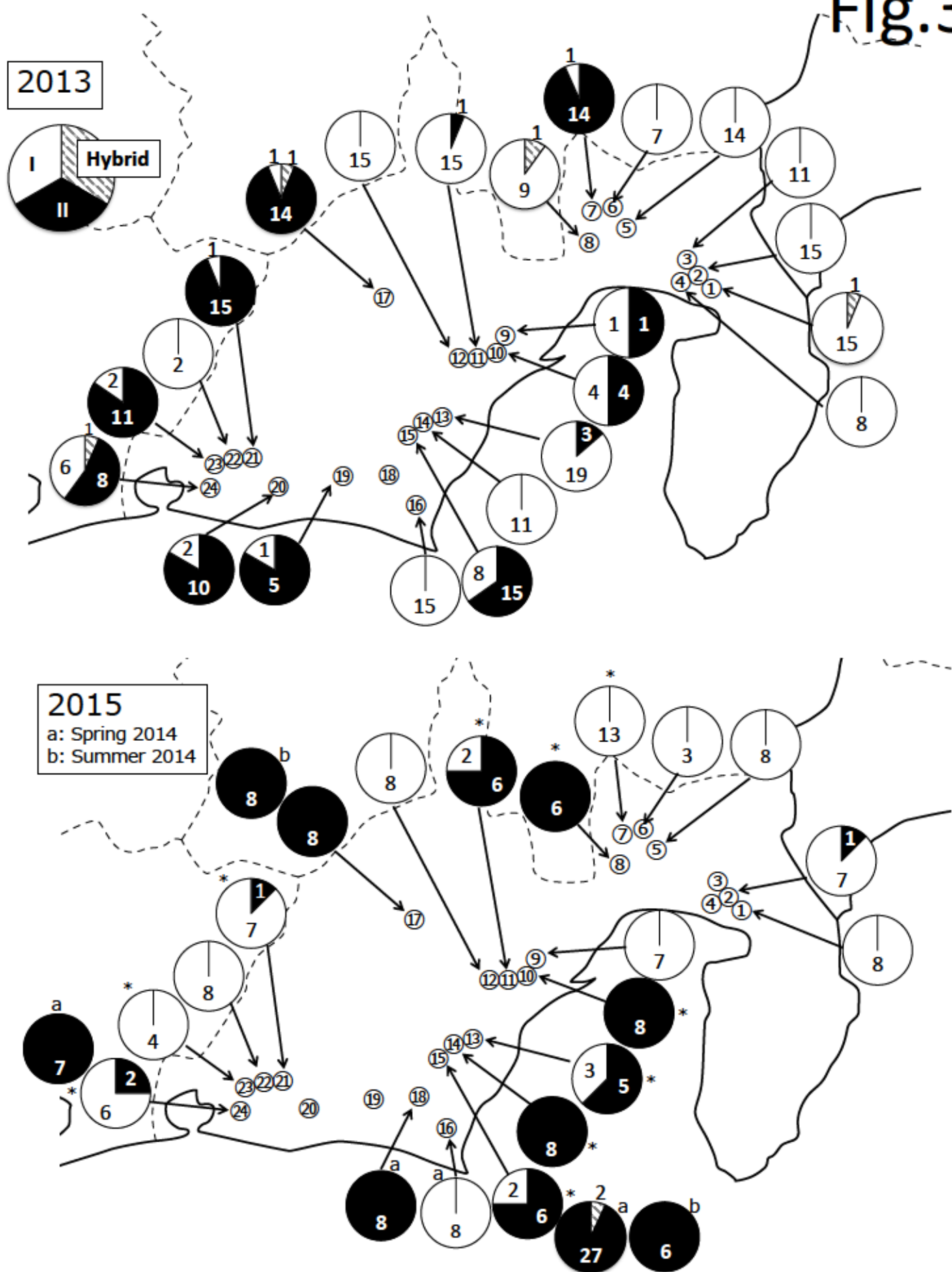
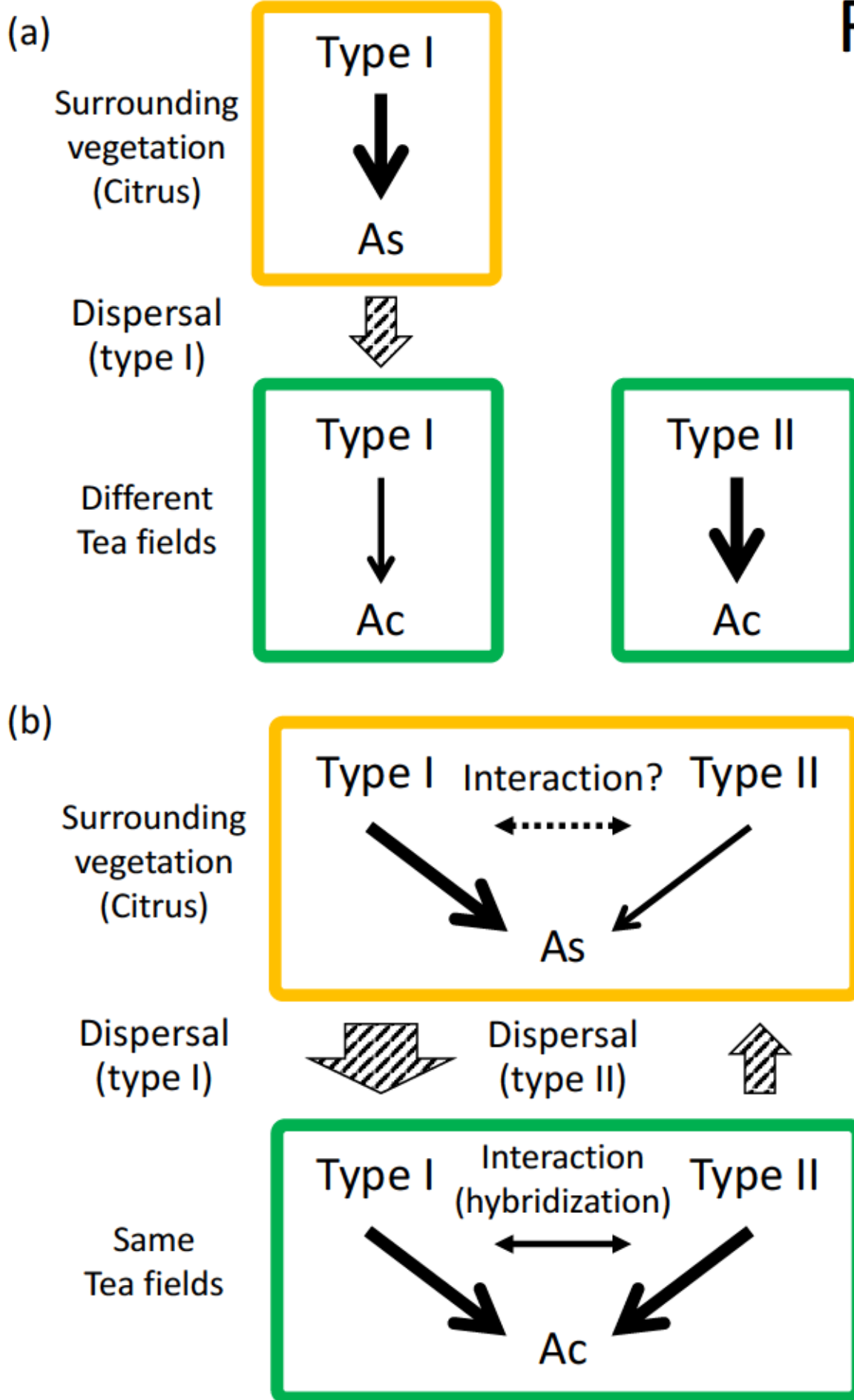




Fig.4



**Table 1.** *Encarsia smithi* used for DNA sequencing of the nuclear rDNA ITS region

Phylogroup	Code <sup>a</sup>	City	Prefecture	Year	Host	Host plant	<i>n</i>
I	SHZ2	Okitsu	Shizuoka	2010	<i>Aleurocanthus spiniferus</i>	<i>Citrus unshiu</i>	3
I	EHI	Seiyo	Ehime	2010	<i>A. spiniferus</i>	<i>Ci. unshiu</i>	3
I	OKI	Gesashi	Okinawa	2011	<i>A. spiniferus</i>	<i>Caesalpinia crista</i>	3
II	GIF	Ogaki	Gifu	2010	<i>Aleurocanthus camelliae</i>	<i>Camellia sinensis</i>	4
II	MIE	Kameyama	Mie	2009	<i>A. camelliae</i>	<i>Ca. sinensis</i>	4
II	NAR	Nara	Nara	2010	<i>A. camelliae</i>	<i>Ca. sinensis</i>	4

<sup>a</sup> Uesugi et al. (2016a)

**Table 2.** Specific primer pairs and PCR product size in *Encarsia smithi*

Phylogroup	Name	Sequence (5'-3')	Size (bp)
I	ITS1-EsI-F	CACGCAACGTTTTAAACTTTATAC	993
	ITS2-EsI-R	CAAGTCGACCGCGATTAGTC	
II	ITS1-EsII-F	GAAACTTGATACAGAAATTCG	890
	ITS2-EsII-R	CTTAAAATTTCTCAGAAAGAGG	

**Table 3.** Sample locations and numbers of *Encarsia smithi* analyzed (host - host plant: *Aleurocanthus camelliae* - tea) in this study

No. <sup>a</sup>	City or Town	Jul.-Aug, 2013		Apr.-May, 2014		Aug., 2014		Jul., 2015	
		F <sup>b</sup>	M <sup>c</sup>	F	M	F	M	F	M
1	Numazu_1	16						5	3
2	Numazu_2	15						5	3
3	Numazu_3	11							
4	Numazu_4	5	3						
5	Fujinomiya_1	14						8	
6	Fujinomiya_2	2	5					2	1
7	Fujinomiya_3	15						2	11
8	Fujinomiya_4	10						5	1
9	Shizuoka_1	1	1					6	1
10	Shizuoka_2	6	2					8	
11	Shizuoka_3	16						3	5
12	Shizuoka_4	15						8	
13	Fujieda	22						8	
14	Shimada_1	11						8	
15	Shimada_2	23		29		6		8	
16	Makinohara	15		8					
17	Kawanehoncho	16				8		8	
18	Kikugawa			8					
19	Kakegawa	2	4						
20	Iwata	3	9						
21	Hamamatsu_1	15	1					2	6
22	Hamamatsu_2		2						8
23	Hamamatsu_3	12	1						4
24	Hamamatsu_4	10	5	7				8	
15	Shimada_2								1 <sup>d</sup>

<sup>a</sup> Numbers preceding name of city or town correspond to those in Figure 3.

<sup>b</sup> F: Female.

<sup>c</sup> M: Male.

<sup>d</sup> Obtained from the host *Aleurocanthus spiniferus* infesting a citrus leaf.