

## REVIEW

# Interaction between *Torymus sinensis* (Hymenoptera: Torymidae) and *T. beneficus*, Introduced and Indigenous Parasitoids of the Chestnut Gall Wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae)

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### Abstract

*Torymus sinensis* is a parasitoid wasp that was introduced from China to Japan to control the invasive chestnut gall wasp *Dryocosmus kuriphilus*. Interaction between *T. sinensis* and the indigenous parasitoid *T. beneficus* has been of interest since *T. sinensis* was first released in chestnut orchards, as hybridization between them might impede the success of biological control by the introduced parasitoid. Such apprehensions disappeared with the drastic decrease in damage caused by *D. kuriphilus*. However, the emergence of morphologically intermediate individuals between them after the introduction of the parasitoid has triggered renewed interest in hybridization, specifically on the non-target effects of biological control. In this review, the interaction between both species, specifically hybridization, species composition and species displacement, is surveyed. Molecular markers are used, not only because *T. sinensis* and *T. beneficus* are similar morphologically, but also because the latter has two emergence strains (early- and late-spring). Surveys show that 1) *T. sinensis* has displaced both emergence strains of *T. beneficus*; 2) hybridization between *T. sinensis* and early-spring *T. beneficus* occurs at a low frequency ( $\leq 1\%$ ); but that 3), hybrid  $F_1$ s between *T. sinensis* and late-spring *T. beneficus* occur at a much higher frequency ( $\leq 20\%$ ). A study of phylogenetic relationships indicates that the late-spring *T. beneficus* is closer to the different species *T. sinensis* than the early-spring *T. beneficus*. This can partly explain why *T. sinensis* hybridizes more readily with the late-spring *T. beneficus* than with the early-spring *T. beneficus*.

**Discipline:** Plant protection

**Additional key words:** classical biological control, displacement, hybridization, molecular marker, non-target effect

## Introduction

The chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu, thought to have invaded from China in the 1940s, is a serious pest affecting chestnut trees in Japan. Although the indigenous parasitoid *Torymus beneficus* Yasumatsu et Kamijo was experimentally released in chestnut orchards<sup>16</sup>, it was unable to suppress *D. kuriphilus* populations. The parasitoid *Torymus sinensis* Kamijo was then imported from China into Japan and released at 64 sites from 1982 to 1999<sup>14</sup>. This introduced parasitoid

very effectively reduced the damage caused by *D. kuriphilus*<sup>5,10,13</sup>. This project is regarded as one of the most famous and successful cases of classical biological control in Japan. However, the ecological characteristics of *T. sinensis* and *T. beneficus* remain poorly understood, partly due to the difficulty of rearing them in the laboratory and, as mentioned below, that of discriminating morphologically between them. As for *T. beneficus*, for example, it is not yet clear what gall insects they exploited before the invasion of *D. kuriphilus* in Japan; *T. sinensis* is thought to be a specialist parasitoid of *D. kuriphilus*<sup>9</sup>.

Interaction between *T. sinensis* and *T. beneficus* has

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attracted attention since the first release of *T. sinensis* in 1982: their morphological and ecological characteristics are so similar that researchers were concerned that hybridization between them might impair the effectiveness of biological control by the introduced parasitoid, e.g. through the increase in sterile individuals. Such apprehensions subsided in the face of evidence that damage by *D. kuriphilus* plummeted in chestnut orchards<sup>6</sup>. However, the appearance of individuals morphologically intermediate between *T. sinensis* and *T. beneficus* was reported in the 1990s<sup>1,7</sup>, which sparked a new interest in hybridization, specifically the non-target effects of biological control.

Recent progress in the use of molecular markers has elicited a better understanding of insect interactions, especially hybridization, between closely-related species that are morphologically very similar. This also applies for the case of *T. sinensis* and *T. beneficus*<sup>17</sup>. Only adult females of both species can be discriminated empirically, based on a slight difference in either the length of the ovipositor or the ratio of the length of the ovipositor sheath to the thorax; but the males cannot be empirically discriminated<sup>7,11,21</sup>. Furthermore, because *T. beneficus* has two emergence strains, namely early-spring and late-spring<sup>8,11</sup>, molecular markers could provide a more detailed insight into interactions between these two species/strains.

In this review, I will discuss the interaction between *T. sinensis* and *T. beneficus*, especially hybridization, species composition and species displacement, as revealed by the use of molecular markers.

## Molecular markers for *T. sinensis* and both emergence strains of *T. beneficus*

### 1. Isozyme markers

When morphologically intermediate individuals between both *Torymus* parasitoids appeared in the field, isozyme (or allozyme) markers were initially used to discriminate them. In *T. sinensis* and *T. beneficus* (including both emergence strains), a total of 25 isozymes were tested for available markers, only three of which (malic enzyme, adenylic acid, and aspartate aminotransferase) proved usable to discriminate between early-spring *T. beneficus* and the other parasitoid species/strain (*T. sinensis* and late-spring *T. beneficus*)<sup>2,3</sup> (Noda et al., unpublished data). However, even these three isozymes cannot discriminate between *T. sinensis* and the late-spring *T. beneficus*. Hybridization between *T. sinensis* and the early-spring *T. beneficus* was therefore analyzed using an isozyme (malic enzyme), focusing on sites where the early-spring *T. beneficus* dominated.

### 2. DNA markers

Later, as replacing malic enzyme, the internal transcribed spacer 2 (ITS2) of the rRNA coding region of nuclear DNA and the cytochrome oxidase subunit I (COI) region of mtDNA were developed as discrimination markers between *T. sinensis* and *T. beneficus*<sup>18,20</sup>. Fragment analysis, which makes it possible to detect slight differences in the length of PCR products, is used as the ITS2 marker<sup>18</sup>, while PCR-RFLP is used as the COI marker<sup>20</sup>. Both ITS2 and COI are superior to malic enzyme in their ability to discriminate, especially when used together. Neither, however, can clearly analyze hybridization of the *Torymus* parasitoids, since (1) the ITS2 marker cannot discriminate between some *T. sinensis* individuals and the late-spring *T. beneficus*; and (2) the COI marker on mtDNA is maternally inherited.

Subsequently, the internal transcribed spacer 1 (ITS1) of the rRNA coding region of nuclear DNA was developed<sup>23</sup>. Now, three kinds of allele-specific PCR, using four designed specific primers, make it possible to distinguish *Torymus* species, the emergence strains of *T. beneficus*, and F<sub>1</sub> (first filial) individuals.

## Hybridization between *T. sinensis* and *T. beneficus*

### 1. The early-spring *T. beneficus*

The frequency of the malic enzyme genotype of the F<sub>1</sub> between *T. sinensis* and the early-spring *T. beneficus* was 13% (n = 15) in Tsukuba, Ibaraki Prefecture in 1992<sup>3</sup>, and 8% (n = 93) on the Oki Islands of Shimane Prefecture in 1996<sup>15</sup>. The F<sub>1</sub> genotype frequency was 1% (n = 821) in specimens collected from 20 populations at 11 sites in the Tokyo Metropolis and Nagano, Ibaraki, Saitama, and Gunma prefectures from 1991 to 1995<sup>19</sup>. These studies demonstrate that hybridization had, in fact, occurred between *T. sinensis* and the early-spring *T. beneficus*, although its frequency was much lower than we expected.

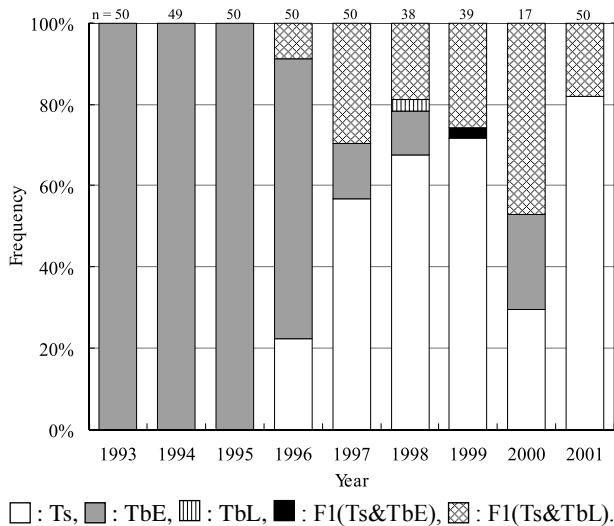
Adult female specimens (n = 418) collected from Obuse, Nagano Prefecture during 1993-2001, were analyzed using both ITS2 and COI<sup>20</sup>. From 1993 to 1995, all specimens were the early-spring *T. beneficus* (Fig. 1). *Torymus sinensis* was first detected in 1996; the early-spring *T. beneficus* eventually appeared to be displaced by *T. sinensis*. Simultaneously with this displacement, we found only one individual descendant of the F<sub>1</sub> hybrid; it had both genotypes of *T. sinensis* in ITS2 and one of the early-spring *T. beneficus* in COI. Hybridization between them was thus rare, and thus did not appear to be the main factor driving the displacement of the early *T. beneficus*. *Torymus sinensis* excels early-spring *T. beneficus* in reproductive ability<sup>12</sup>.

The larvae of *Torymus* parasitoids (n = 448), collected from the same sampling site described as above (Obuse) from 1996 to 1998 and in 2000, were also analyzed using ITS2 (Fig. 2). The frequency of the F<sub>1</sub> genotype between *T. sinensis* and the early-spring *T. beneficus* exceeded that at the adult stage, suggesting that (1) hybridization between *T. sinensis* and the early-spring *T. beneficus* did in fact occur, but that (2) most of these F<sub>1</sub> larvae did not survive to the adult stage.

**2. The late-spring *T. beneficus***

From the samples above (adult female specimens (n = 418) collected from Obuse, Nagano Prefecture during 1993-2001), there were also 10 individuals with both the genotype of *T. sinensis* in ITS2 and that of the late-spring *T. beneficus* in COI<sup>20</sup>. This is the first report of hybridization occurring in the field between *T. sinensis* and the late-spring *T. beneficus*. It also suggests that the late-spring *T. beneficus* hybridized with *T. sinensis* elsewhere and that the hybrids or their descendants migrated to the research site, since originally only the early-spring *T. beneficus* had been present. These results led to research into possible interaction with late-spring *T. beneficus*. Reanalysis using ITS1 showed 49 individuals to be hybrids (F<sub>1</sub> and their descendants) with the late-spring *T. beneficus* (Fig. 1).

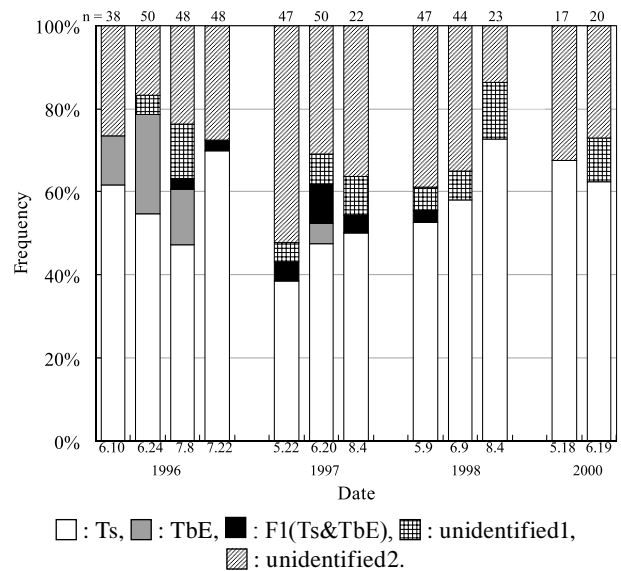
Adult female *Torymus* parasitoids (n = 200), collected from Tobu, Nagano Prefecture from 1993-1996, were



**Fig. 1. Annual changes in the frequency of types of nDNA ITS2, ITS1 and mtDNA COI in adult female *Torymus* parasitoids in Obuse, 1993-2001**

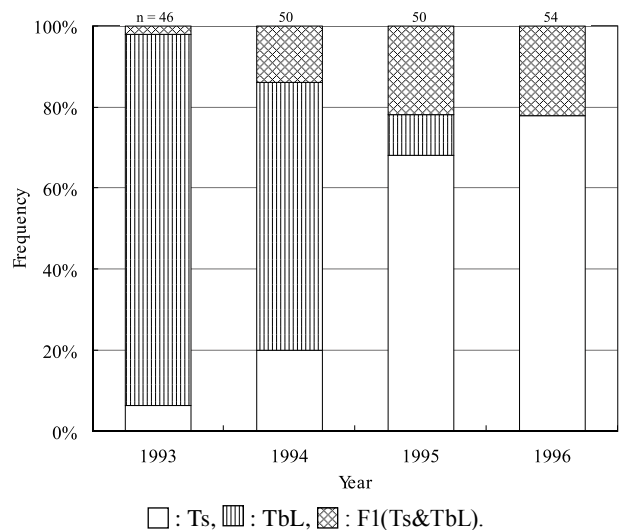
Ts: *T. sinensis*; TbE: early-spring *T. beneficus*; TbL: late-spring *T. beneficus*; F1(Ts&TbE): hybrid between *T. sinensis* and early-spring *T. beneficus*; F1(Ts&TbL): hybrid between *T. sinensis* and late-spring *T. beneficus*<sup>20</sup>.

analyzed using ITS1<sup>21</sup>. Increasing numbers of F<sub>1</sub> hybrids were detected every year from 1993 to 1996, reaching 22% (Fig. 3). It appears that *T. sinensis* can hybridize more readily with the late-spring *T. beneficus* than with the early-spring *T. beneficus*. The morphological indices of F<sub>1</sub> hybrids identified using the ITS1 marker ranged be-



**Fig. 2. Annual changes in the frequency of ITS2 type in larvae of *Torymus* parasitoids in Obuse, 1996-2000**

Ts: *T. sinensis*; TbE: early-spring *T. beneficus*; F1(Ts&TbE): hybrid between *T. sinensis* and early-spring *T. beneficus*; unidentified 1: *T. sinensis* or late-spring *T. beneficus*; unidentified 2: *T. sinensis* or hybrid between *T. sinensis* and late-spring *T. beneficus*.



**Fig. 3. Annual changes in frequency of ITS1 type in adult females of *Torymus* parasitoids in Tobu, 1993-1996**

Ts: *T. sinensis*; TbL: late-spring *T. beneficus*; F1(Ts&TbL): hybrid between *T. sinensis* and the late-spring *T. beneficus*<sup>21</sup>.

tween indices of *T. sinensis* and the late-spring *T. beneficus*, with considerable overlap. Simultaneously with the occurrence of the F<sub>1</sub> hybrids, the indigenous late-spring *T. beneficus* was rapidly displaced by *T. sinensis*. These facts suggest that hybridization is a more significant factor in the displacement of the late-spring *T. beneficus* by *T. sinensis* than in the displacement of the early-spring *T. beneficus*.

### Phylogenetic relationship

A phylogenetic tree was constructed to examine the genetic relationships between *T. sinensis* and *T. beneficus* (early- and late-spring strains) collected from various places in Japan, China, and South Korea, using mtDNA COI sequences (1129bp)<sup>17</sup>(Fig. 4). There were two distinct groups in the phylogenetic tree obtained: A and B. Group A consisted of two subgroups: A1 and A2 (Fig. 5). *Torymus sinensis* belonged to Subgroup A1 within Group A, while Subgroup A2 consisted of late-spring *T. beneficus*. Early-spring *T. beneficus* belonged to Group B. These results show that the late-spring *T. beneficus* is closer to the different species *T. sinensis* than the early-spring *T.*

*beneficus*. This may partly explain why *T. sinensis* hybridizes more easily with the late-spring *T. beneficus* than with the early-spring *T. beneficus*.

### Further remarks

Recent progress in the use of molecular markers has revealed complicated interactions among *Torymus* species/strains. For example, our study has shown their unexpected interactions in a Japanese chestnut orchard in Obuse of Nagano Prefecture where *T. koreanus*, endemic in Korea, was recently discovered<sup>4</sup>; at the site, early-spring and late-spring *T. beneficus* were almost completely displaced by *T. sinensis* for several years, whereas this was not the case for *T. koreanus* (Table 1). Further progress in molecular phylogenetic studies will shed light on the underlying mechanisms of the complicated interactions among *Torymus* species/strains and the patterns of non-target effects of classical biological control.

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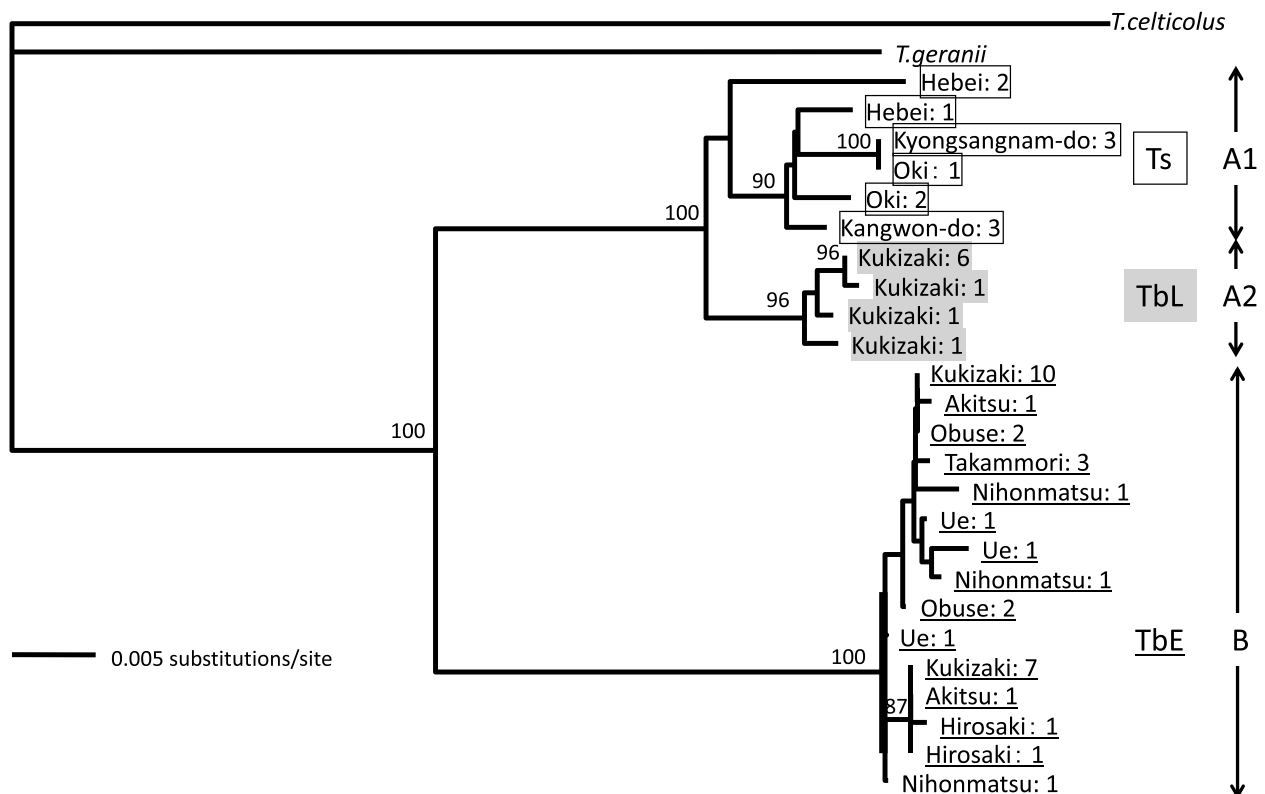


Fig. 4. Collection sites of *Torymus* spp. to analyze the phylogenetic relationship

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**Fig. 5.** Phylogenetic tree inferred from 1,129-bp sequences of the mitochondrial COI gene of *Torymus* spp. using the neighbor-joining method

Ts: *Torymus sinensis*; TbE: early-spring *T. beneficus*; TbL: late-spring *T. beneficus*. Each OTU (operational taxonomic unit) is denoted by the collection site followed by the number of specimens. *Torymus geranii* and *T. celticolus* was used as outgroups. Bootstrap probabilities (500 replicates) are provided on each branch if they exceed 70%. The scale of distances is shown under the tree<sup>17</sup>.

**Table 1.** Composition of *Torymus* parasitoids collected from galls on *Dryocosmus kuriphilus* in Obuse<sup>22</sup>

Year	n	<i>Torymus</i> species <sup>a</sup> (%)					
		Tk	TbE	TbL	Ts	F <sub>1</sub> (Ts&TbE)	F <sub>1</sub> (Ts&TbL)
1992	24	20.8	58.3	20.8	0.0	0.0	0.0
1994	24	12.5	54.2	16.7	8.3	4.1	4.1
1996	24	16.7	8.3	0.0	58.3	0.0	16.7

a Tk: *Torymus koreanus*; TbE: early-spring *T. beneficus*; TbL: late-spring *T. beneficus*; Ts: *T. sinensis*; F<sub>1</sub>(Ts&TbE): F<sub>1</sub> hybrid between *T. sinensis* and early-spring *T. beneficus*; F<sub>1</sub>(Ts&TbL): F<sub>1</sub> hybrid between *T. sinensis* and late-spring *T. beneficus*.

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