



# Comparisons of parasitism and developmental properties of *Trissolcus japonicus* (Hymenoptera: Scelionidae) reared on eggs of three species of fruit-piercing stink bugs

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## Abstract

An egg parasitoid, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), was reared on eggs of *Plautia stali* scott (Hemiptera: Pentatomidae), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), and *Glaucias subpunctatus* (Walker) (Hemiptera: Pentatomidae), and parasitism rates and developmental properties were compared to clarify their suitability as hosts. The parasitoids successfully emerged from more than 90% of *P. stali* or *G. subpunctatus* eggs, whereas a lower rate of *T. japonicus* emerged from the eggs of *H. halys*. The developmental period of *T. japonicus* in the eggs of *H. halys* was longer than in the other two species, and the body size of *T. japonicus* that emerged from the eggs of *H. halys* was also larger than from the other two species. From these results, factors related to parasitism and developmental properties were discussed.

**Keywords** Developmental properties · Fruit-piercing stink bugs · Host egg size · Parasitism · *Trissolcus japonicus*

## Introduction

Fruit-piercing stink bugs are important pests that cause damage to various fruits (Tsutsumi 2001). Forty-five species of fruit-piercing stink bugs have been reported in Japan (Japanese Society of Applied Entomology and Zoology 2006), and the principal species are *Plautia stali* scott (Hemiptera: Pentatomidae), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), and *Glaucias subpunctatus* (Walker) (Hemiptera: Pentatomidae) (Hasegawa and Umeya 1974; Kiritani 2007). They have been controlled solely by chemical spraying (Funayama 2004; Suzuki 2005); however, continuous spraying of chemicals due to frequent fruit-piercing stink bugs increases the labor and economic burden of producers. It is also responsible for the resurgence of spider mites and scale insects due to the reduction of their natural enemies in orchards (Tsutsumi 2003). Therefore, it is desirable to establish control methods that offer an alternative to spraying chemicals, and biological control by natural enemies

has been attracting attention as one of the methods (Goto and Adachi 2004).

Most species of the genus *Trissolcus* Ashmead (Hymenoptera: Scelionidae) are egg parasitoids of bugs of the superfamily Pentatomoidea (Talamas et al. 2015; Talamas et al. 2017). *Trissolcus plautiae* (Watanabe) is the dominant species parasitizing egg masses of *P. stali* in the field, and their parasitism rate reaches more than 90% from late August to early September (Yamada and Miyahara 1979). Therefore, *T. plautiae* has been expected as a classical biological control agent for *P. stali* in Japan (Goto and Adachi 2004). *Trissolcus mitsukurii* (Ashmead) is the commonest species that can parasitize eggs of the southern green stink bug *Nezara viridula* (L.) (Hemiptera: Pentatomidae) in Japan (Kiritani and Hokyo 1962), and it is also known as a parasitoid of *H. halys* (Arakawa and Namura 2002). *Trissolcus japonicus* (Ashmead) is distributed in Japan and it has been reported to parasitize eggs of *P. stali* and *H. halys* in the field (Matsuo et al. 2014). Matsuo et al. (2016) collected *T. japonicus* that emerged from egg masses of *G. subpunctatus* in Japan and reported that *T. japonicus* is the dominant species among egg parasitoids of *G. subpunctatus*. From these reports, *T. japonicus* affects the population of these stink bugs. However, there is no report simultaneously comparing the suitability of fruit-piercing stink bugs as a host for *T. japonicus*.

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In this study, eggs of three species of bugs were supplied for parasitization by *T. japonicus*, and parasitism rates and developmental properties were compared to clarify their suitability as hosts.

## Materials and methods

### Insects

A stock culture of *T. japonicus* was established from adults collected in Ogi city, Saga prefecture in August 2014 using egg masses of *G. subpunctatus* (Kuki et al. 2019). Laboratory colonies were maintained in 50 mL plastic tubes (3.0 cm diameter and 11.5 cm height) placed in incubators (MIR-153; Sanyo Electric Co., Ltd., Tokyo, Japan) set at  $25 \pm 1$  °C and  $60 \pm 10\%$  RH with a 16L:8D photoperiod. A newly laid egg mass of *G. subpunctatus* was supplied for oviposition and a drop of undiluted honey was applied to the inner wall every 5 days.

Laboratory stock cultures of *P. stali*, *H. halys*, and *G. subpunctatus* originated and were continuously refilled from Shizuoka prefecture, Akita prefecture, and Mie prefecture, respectively. Adults were continuously reared on raw peanuts (*Arachis hypogaea* L.), raw soybeans (*Glycine max* L., only for *P. stali*), and water containing 0.05% L-ascorbic acid sodium salt and 0.025% L-cysteine hydrochloride monohydrate in glass petri dishes (123 mm diameter  $\times$  23 mm height) placed in incubators set at  $25 \pm 1$  °C and  $60 \pm 10\%$  RH with a 16L:8D photoperiod. Food and water were replaced every 3–4 days. Newly laid egg masses were collected and transferred to nymph plastic containers (135 mm diameter  $\times$  76 mm height) with a 3 cm diameter, mesh-covered hole containing a *Morella rubra* Sieb. et Zucc. twig (Fagales: Myricaceae) (Shimizu 2010) under the same environmental conditions. Newly emerged adults were collected from the cages and male and female pairs were evenly distributed among adult rearing cages to obtain egg masses.

### Parasitism rate and developmental period of *T. japonicus*

Newly laid (0–24 h) egg masses of *P. stali* (containing 14 eggs), *H. halys* (28 eggs), and *G. subpunctatus* (14 eggs) were used in the experiments. One egg mass laid on filter paper was stuck on the inner wall of a 50 mL plastic tube and a drop of undiluted honey was also applied to the inner wall. One female egg parasitoid (3–5 days old) was released in the tubes and kept at  $25 \pm 1$  °C and  $60 \pm 10\%$  RH with a 16L:8D photoperiod. Most hymenopterous parasitoids are haplodiploid and haploid males develop from unfertilized eggs and diploid females from fertilized ones (Colazza and Wajnberg 1998). Therefore, in this study, egg masses

exposed to a mated female provided female egg parasitoids except for a few males. On the other hand, egg masses exposed to virgin females provided male egg parasitoids. Male egg parasitoids obtained from mated females were also used for experiments. After 6 h exposure, the egg parasitoids were removed, and parasitized egg masses were then kept in incubators under the same conditions. Egg masses were checked every 12 h and the time for an egg parasitoid to emerge from an egg was recorded. Observation of the eggs was continued until all egg parasitoids had emerged, and the numbers of eggs that fruit-piercing stink bug nymphs or egg parasitoid adults emerged from were counted. Eggs not showing emergence were dissected under a microscope (SZX16; Olympus Co., Tokyo, Japan) and classified into undeveloped parasitoids (egg parasitoid adult dead in egg) and dead eggs (undefined content).

### Body size of *T. japonicus*

Emerged egg parasitoids were supplied a drop of undiluted honey and kept under the same conditions, and female and male egg parasitoids that emerged from each host egg were randomly selected. Egg parasitoids (3 days old) in the tubes were killed in a freezer (MDF-U538D; Panasonic Healthcare Co., Ltd., Tokyo, Japan) and kept until measurement. The head and forewings of the dead parasitoids were removed from the body under a microscope and photographed with a camera (HD Lite 1080P; RelyOn, Ltd., Tokyo, Japan) attached to the microscope. The head and forewings were measured using the image processing program ImageJ (Rasband 1997–2018) with reference to a micrometer similarly photographed.

### Host egg size

For each host species, 25 eggs from 5 egg masses (5 eggs per egg mass) were randomly selected. After careful division by tweezers, each egg was photographed from above and the side under the microscope, and then its diameter and height were measured in the same way as the body size of *T. japonicus*.

### Statistical analyses

The rates of parasitized eggs were analyzed by logistic regression analysis to estimate the effect of host species. Then if necessary, pairwise comparisons using the *G* test with Bonferroni correction were performed. The data of the developmental period, body size of *T. japonicus*, and host egg size were first examined using Bartlett's test for homogeneity of variances. When significant differences were indicated, the data were analyzed using the Kruskal–Walis test followed by Dwass–Steel–Crichtlow–Fligner multiple

**Table 1** Parasitism of *T. japonicus* reared on eggs of three species of fruit-piercing stink bugs

Host species	Total no. of eggs ( <i>n</i> )	Parasitoids emerged (%)	Undeveloped parasitoids (%)	Dead eggs (%) (undefined content)	Nymphs emerged (%)
<i>Plautia stali</i>	126	99.2 b	0.8 a	0.0	0.0
<i>Halyomorpha halys</i>	420	22.1 a	2.9 a	58.1 b	16.9 b
<i>Glaucias subpunctatus</i>	294	97.3 b	1.0 a	1.4 a	0.3 a

Values followed by the same letter within a column are not significantly different ( $p < 0.05/3$ ) by the *G* test with Bonferroni correction after logistic regression analysis ( $p < 0.05$ )

**Table 2** Developmental periods of *T. japonicus* reared on eggs of three species of fruit-piercing stink bugs

Host species	<i>n</i>		Developmental periods (days) <sup>A</sup>	
	Female	Male	Female	Male
<i>Plautia stali</i>	50	74	12.2 ± 0.08 b <sup>B</sup>	10.5 ± 0.03 a <sup>B</sup>
<i>Halyomorpha halys</i>	60	41	14.1 ± 0.18 c	13.3 ± 0.23 b
<i>Glaucias subpunctatus</i>	133	154	11.9 ± 0.02 a	10.4 ± 0.03 a

<sup>A</sup>Mean ± SE

<sup>B</sup>Values followed by the same letter within a column are not significantly different by Dwass–Steel–Crichtlow–Fligner multiple pairwise comparisons tests ( $p < 0.05$ )

pairwise comparisons tests. When significant differences were not indicated, the data were analyzed using one way ANOVA followed by Tukey–Kramer multiple comparisons tests. All statistical analyses were performed by SAS university edition Version 3.8 (SAS Institute 2018).

## Results

### Parasitism rate and developmental period of *T. japonicus*

The parasitoids successfully emerged from more than 90% of *P. stali* or *G. subpunctatus* eggs, whereas a lower rate of *T. japonicus* emerged from the eggs of *H. halys* (Table 1).

When *T. japonicus* was supplied with eggs of *H. halys*, the numbers of dead eggs and successfully emerging nymphs of host were significantly higher. The developmental periods of both females and males in the eggs of *H. halys* were significantly longer than those of other fruit-piercing stink bugs (Table 2). The developmental periods of female egg parasitoids in the eggs of *P. stali* and *G. subpunctatus* were significantly different; however, the developmental periods of male egg parasitoids in the eggs of *P. stali* and *G. subpunctatus* were not significantly different.

### Body size of *T. japonicus* and host egg size

The head width and forewing length of both females and males were significantly different depending on the host species (Table 3). The head width and forewing length of egg parasitoids emerging from *H. halys* eggs were significantly the largest among egg parasitoids emerging from the three fruit-piercing stink bugs, followed by those emerging from *G. subpunctatus* and *P. stali*.

The diameter and height of host eggs were significantly different among the three host species (Table 4). The diameter of *H. halys* was significantly the longest, followed by those of *G. subpunctatus* and *P. stali*. The heights of *H. halys* and *G. subpunctatus* were not significantly different, with *P. stali* being significantly the shortest.

**Table 3** Body size of *T. japonicus* reared on eggs of three species of fruit-piercing stink bugs

Host species	<i>n</i>		Head width (mm) <sup>A</sup>		Wing length (mm) <sup>A</sup>	
	Female	Male	Female	Male	Female	Male
<i>Plautia stali</i>	130	136	0.603 ± 0.001 a <sup>B</sup>	0.565 ± 0.001 a <sup>B</sup>	0.998 ± 0.002 a <sup>B</sup>	1.021 ± 0.002 a <sup>C</sup>
<i>Halyomorpha halys</i>	27	72	0.688 ± 0.003 c	0.649 ± 0.002 c	1.157 ± 0.005 c	1.191 ± 0.003 c
<i>Glaucias subpunctatus</i>	107	145	0.671 ± 0.001 b	0.618 ± 0.001 b	1.139 ± 0.002 b	1.146 ± 0.002 b

<sup>A</sup>Mean ± SE

<sup>B</sup>Values followed by the same letter within a column are not significantly different by Dwass–Steel–Crichtlow–Fligner multiple pairwise comparisons tests ( $p < 0.05$ )

<sup>C</sup>Values followed by the same letter within a column are not significantly different by Tukey–Kramer multiple comparisons tests ( $p < 0.05$ )

**Table 4** Host egg size ( $n=25$ )

Host species	Diameter (mm) <sup>A</sup>	Height (mm) <sup>A</sup>
<i>Plautia stali</i>	0.993 ± 0.006 a <sup>B</sup>	1.112 ± 0.007 a <sup>C</sup>
<i>Halyomorpha halys</i>	1.281 ± 0.008 c	1.395 ± 0.009 b
<i>Glaucias subpunctatus</i>	1.168 ± 0.005 b	1.368 ± 0.008 b

<sup>A</sup>Mean ± SE

<sup>B</sup>Values followed by the same letter within a column are not significantly different by Dwass–Steel–Crichtlow–Fligner multiple pairwise comparisons tests ( $p < 0.05$ )

<sup>C</sup>Values followed by the same letter within a column are not significantly different by Tukey–Kramer multiple comparisons tests ( $p < 0.05$ )

## Discussion

In this study, it was shown that parasitism and developmental properties of *T. japonicus* differ depending on the species of host egg. Previous studies also reported that parasitism and developmental periods in several species of *Trissolcus* were different depending on the species of host (Botch and Delfosse 2018; Kivan and Kilic 2002, 2004, 2005). Also, positive correlations were reported between the host egg size and parasitoid body size in other *Trissolcus* species (Arakawa et al. 2004; Botch and Delfosse 2018). Those previous reports strongly support the validity of the present study. Female *T. japonicus* reared on larger host eggs can produce more offspring as a consequence (Botch and Delfosse 2018). The relationship between the host egg size and fecundity of emerging parasitoids was also reported in other Scelionidae (Abram et al. 2016b; Arakawa et al. 2004). If this is also the case for *T. japonicus*, individuals emerging from eggs of *H. halys* should have higher reproductive capacities; therefore, *H. halys* might be a suitable host for *T. japonicus*.

Although the rates of *T. japonicus* parasitizing *H. halys* eggs under experimental conditions were previously reported to be from 50 to 90% (Haye et al. 2020; Hedstrom et al. 2017), lower success rates (25.0% for parasitism and 22.1% for emergence, respectively) were observed in this study. Over 50% of the provided *H. halys* eggs produced neither adult parasitoids nor host nymphs, as shown in Table 1. Egg parasitoids are known to induce host egg abortion even when the parasitism is not successful (Abram et al. 2016a). Since almost all of *H. halys* eggs hatch successfully in our laboratory conditions, we suggest this could also be the case in our study. Similarly, host egg abortion has been well documented in other Scelionidae (Abram et al. 2016a; Konopka et al. 2018, 2020). We consider two reasons why failure was more marked in this study. First, it may be due to a difference in the genetic background of *T. japonicus*. The interactions between parasitoids and hosts are influenced by various factors; such as physiological or nutritional condition of host,

venom injected by adult parasitoid at oviposition, and teratocytes released by hatched larvae of parasitoid (Beckage and Gelman 2004; Konopka et al. 2020; Pennacchio and Strand 2006; Volkoff and Colazza 1992). Thus, we suggest that some differences in abovementioned factors might have induced different adaptations to *H. halys* eggs in the present study. Furthermore, since there is some intra-specific variation in the capacity to abort host eggs (Abram et al. 2016a), we also suggest that investigated populations that have different genetic background have caused the different adaptations. Further investigations of the intra-specific differences in parasitism and capacity to resist the host.

Another possible reason is the effect of continuous breeding. Botch and Delfosse (2018) reported that *T. japonicus* reared on eggs of species other than *H. halys* had lower reproductive success on *H. halys* eggs. The stock culture of *T. japonicus* in this study was maintained on egg masses of *G. subpunctatus*, not on *H. halys*, for a long time. Even if the stock culture has the same genetic background, it is possible that adaptation to *H. halys* was reduced due to continuous breeding on eggs of *G. subpunctatus*. Further investigation is also required to examine the effects of continuous breeding of *T. japonicus*.

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