

Effects of host species on the body size, fecundity, and longevity of *Trissolcus mitsukurii* (Hymenoptera: Scelionidae), a solitary egg parasitoid of stink bugs

Ryo ARAKAWA,* Mirei MIURA and Maki FUJITA

Laboratory of Applied Entomology, Faculty of Agriculture, Kochi University; Nankoku, Kochi 783–8502, Japan

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Abstract

An investigation was conducted to determine whether differences in host size of different species affect the body size, fecundity, and longevity of *Trissolcus mitsukurii*, a solitary egg parasitoid of stink bugs. The eggs of the Pentatomid bugs of *Halyomorpha halys* (large size), *Plautia crossota stali* (middle size), and *Nezara viridula* (small size) were supplied to *T. mitsukurii* and incubated until progeny emerged at $25 \pm 2^\circ\text{C}$ and a photoperiod of LD 16:8. Both male and female parasitoids emerged with larger body sizes when they developed in large host eggs such as *H. halys*. Larger size parasitoids had greater longevity and larger size females showed higher fecundity.

Key words: *Trissolcus mitsukurii*; body size; fecundity; longevity; biological control

INTRODUCTION

The southern green stink bug, *Nezara viridula* (L.), is one of the most important pests of agricultural crops worldwide. *N. viridula* is distributed in the plains of the coastal areas of southwest Japan (Yasunaga et al., 1993). In Kochi Prefecture, *N. viridula* is one of the main pests of pecky rice in the field (Shimomoto, 1998). Under the use of conventional chemical pesticides it is difficult to control the occurrence of nymphs of *N. viridula* hatched from eggs deposited in rice fields after insecticide application (Shimomoto, 1998). Shimomoto (1999) recommended to delay the time of insecticide application. Currently in order to ensure sustainable agriculture of rice fields in Kochi Prefecture we suggest another control method by indigenous natural enemies of *N. viridula*.

The indigenous scelionid wasp, *Trissolcus mitsukurii* (Ashmead), is a major solitary egg parasitoid of *N. viridula* in Japan (Kiritani and Hokyo, 1962; Hokyo and Kiritani, 1963). Kiritani and Hokyo (1962) suggested that *N. viridula* could be controlled if the percent of parasitism in the first generation eggs of *N. viridula* was increased by release of egg parasitoids like *T. mitsukurii*. In this case it is important to use highly effective parasitoids with high reproductive capacity.

T. mitsukurii attacks several species of stink bugs other than *N. viridula* (Hokyo and Kiritani, 1966; Ryu and Hirashima, 1984). In the field we found that *T. mitsukurii* attacks the eggs of the brown marmorated stink bug, *Halyomorpha halys* Stål (Arakawa and Namura, 2002). In the laboratory, *T. mitsukurii* also attacked the eggs of the brown-winged stink bug, *Plautia crossota stali* Scott (Arakawa, unpublished). These stink bugs are not listed as hosts of *T. mitsukurii* in Hokyo and Kiritani (1966) and Ryu and Hirashima (1984). The egg sizes of *H. halys*, *P. crossota stali*, and *N. viridula* clearly differ. When the larva of *T. mitsukurii* develops in the host eggs of different sizes followed by different host species, the body size of adult *T. mitsukurii* may be affected by the host size.

In this study we investigated whether the body size of *T. mitsukurii* parasitizing three different host species affects fecundity and longevity.

MATERIALS AND METHODS

Laboratory culture. Parasitoid cultures of *T. mitsukurii* were derived from female adults that emerged from *H. halys* eggs set in the field at Kochi University, Kochi Prefecture, Japan in 1997. *T. mitsukurii* were maintained on *H. halys* eggs in the laboratory of the Faculty of Agriculture, Kochi

* To whom correspondence should be addressed at: E-mail: arakawar@cc.kochi-u.ac.jp

University. The newly emerged male and female adults of *T. mitsukurii* of the same age were reared in a glass test tube (15 cm length and 1.5 cm diameter) inside the incubator at $25\pm 2^\circ\text{C}$, LD 16:8 and provided with undiluted honey. The females of *T. mitsukurii* aged less than 1 week were transferred to the plastic petri dish (1.5 cm height and 9.0 cm diameter) provided with fresh egg masses (1 d old) of *H. halys* collected from the culture mentioned below for 2 to 5 days. The parasitized eggs were kept in the same dish at $25\pm 2^\circ\text{C}$, LD 16:8 and newly emerged adults were collected.

The laboratory culture of *N. viridula* and *P. crossota stali* originated in Kochi Prefecture in 1998, and *H. halys* originated in Toyama Prefecture, Japan in 1997. These stink bugs were cultured in transparent plastic cups (8 cm height, 11 cm upper diameter and 10 cm lower diameter) while being provided with water, soybeans, peanuts, and sunflower seeds without the husks at $25\pm 2^\circ\text{C}$ and a photoperiod of LD 16:8.

Measurement of host egg size. Five egg masses (less than 48 h after oviposition) of *N. viridula*, *P. crossota*, and *H. halys* reared for successive generations in the laboratory of Kochi University were randomly selected. In total, 25 eggs (five eggs selected randomly from these egg masses) of each species were measured. The eggs of *N. viridula*, *P. crossota*, and *H. halys* are cylindrical or barrel shaped. Width and height of individual host eggs were measured under a microscope equipped with a built-in micrometer.

Body size, fecundity and longevity of the parasitoid. Experiments were conducted by supplying two or three host egg masses (less than 48 h after oviposition) of each host species to three to five parasitoid females aged two to five days. Each egg mass of *N. viridula*, *P. crossota*, and *H. halys* contained about 60, 14, and 28 eggs, respectively. The parasitoid females had no previous oviposition experience.

In this study we did not consider the effect of superparasitism. Three to five females of *T. mitsukurii* were kept in the same petri dish to obtain the next generation for measurements in this study. The female of *T. mitsukurii* demonstrates marking behavior after oviposition and guarding behavior between and after oviposition in a manner similar to other *Trissolcus* spp. (Wilson, 1961; Ohno, 1999). In a closed environment (petri dish) these

behaviors may inhibit superparasitism. Even if multioviposition occurred, the first instar larvae with large mandibles (Telaniform by Clausen (1962)) that hatched earlier attacks the other parasitoid eggs.

Both host egg masses and parasitoids were kept in plastic petri dish (1.5 cm height and 9 cm diameter) with a drop of honey and a piece of moist cotton for 2–3 days at $25\pm 2^\circ\text{C}$ and a photoperiod of LD 16:8. As *T. mitsukurii* tend to avoid oviposition in old aged hosts (more than 6 days at 25°C) (Arakawa, unpublished), the effect of host age on the body size of parasitoids appeared to be negligible.

After removal of the parasitoids, the petri dishes were kept in incubators under the same conditions. The parasitized egg masses were examined daily for parasitoid emergence. Males and females emerged on the same days and were transferred to test tubes (18 cm length and 1.8 cm diameter), separated by host species. Test tubes with more than 50 parasitoids were kept in a refrigerator at -30°C to kill the parasitoids. Twenty females were randomly selected from one test tube and twenty males from 3 to 5 test tubes. The head and right fore wings of the dead parasitoids were removed from the body and mounted to obtain measurements of head width and wing length using the built-in micrometer of the microscope.

In addition, just after parasitoid emergence, 2–3 females each emerged from different host species were randomly selected from the test tubes. This procedure was replicated and a total of 19–20 females per host species were collected. Each individual was dissected in a drop of physiological saline solution placed on a slide glass. After the dissected ovary was completely disassembled in the solution and a drop of methylene blue was added. Only the follicle surrounding the mature egg was stained (Jervis and Copland, 1996). The number of mature eggs and the length (stalk+egg body) and width of mature eggs were determined under the microscope. Other males and females were transferred to test tubes and kept in the incubator at $25\pm 2^\circ\text{C}$ and a photoperiod of LD 16:8 with a drop of honey attached to the inner wall. After 7 days, 2–3 females each emerged from different host species were randomly selected from the test tubes. This procedure was repeated and a total of 15–20 females per host species were col-

lected. All females were dissected to determine fecundity and egg size by the same procedures as above to identify whether oösrption was completed. The remaining males and females were kept in the same incubator to determine their longevity.

Statistical analyses. The data were first examined using Bartlett's test for homogeneity of variances. If significant differences were indicated, the data were analyzed using Steel-Dwass' multiple comparison test. If significant differences were not indicated, the data were analyzed using Tukey's multiple comparison test via ANOVA (Nagata and Yoshida 1997; Zar 1999). The PC software "Excel Statistics, ver 5.0 (2000): Esumi Co. Ltd." was used for the calculations.

RESULTS

Size of host eggs

Table 1 shows the size of the three host eggs. Diameter and length were progressively larger in *N. viridula*, *P. crossota stali*, and *H. halys*. There was a significant difference in both size indexes ($p < 0.05$, Steel-Dwass' test).

Size of adult parasitoids

Table 2 shows the size of *T. mitsukurii* as they emerged from the three different host species. Both size indexes (head width and forewing length) of both males and females differed significantly among parasitoids of the different host species ($p < 0.01$, Tukey's test). Those emerging from *N. viridula* were smallest, while those emerging from *H. halys* were largest.

Fecundity and size of eggs in ovary

Table 3 shows the fecundity of *T. mitsukurii* just after emergence and 7 days after emergence from the three different host species. The post-emergent

Table 1. Size of host eggs ($n=25$)

Species	Diameter (mm) ^a	Height (mm) ^a
<i>Nezara viridula</i>	0.778±0.005 a ^b	1.032±0.004 a
<i>Plautia crossota stali</i>	1.017±0.004 b	1.149±0.005 b
<i>Halyomorpha halys</i>	1.303±0.004 c	1.554±0.006 c

^a Mean±SE.

^b Means followed by the same letter within a column are not significantly different by Steel-Dwass' multiple comparison test ($p < 0.01$).

Table 2. Body size of adult *Trissolcus mitsukurii* emerging from different host species ($n=20$)

Host species	Head width (mm) ^a		Wing length (mm) ^a	
	Male	Female	Male	Female
<i>Nezara viridula</i>	0.556±0.005 a ^b	0.562±0.004 a	1.086±0.007 a	1.124±0.008 a
<i>Plautia crossota stali</i>	0.594±0.006 b	0.618±0.004 b	1.198±0.011 b	1.240±0.004 b
<i>Halyomorpha halys</i>	0.699±0.004 c	0.729±0.004 c	1.400±0.012 c	1.426±0.005 c

^a Mean±SE.

^b Means followed by the same letter within a column are not significantly different by Tukey's multiple comparison test ($p < 0.01$).

Table 3. Fecundity of *Trissolcus mitsukurii* emerged from different host species

Host species	Fecundity (no. of eggs in ovary) ^a			
	<i>n</i>	Just after emergence	<i>n</i>	Age of seven days
<i>Nezara viridula</i>	20	43.9±0.7 a ^b	20	70.1±1.0 a
<i>Plautia crossota stali</i>	19	50.8±1.5 b	15	75.8±1.7 b
<i>Halyomorpha halys</i>	20	45.1±1.3 a	20	90.7±1.2 c

^a Mean±SE.

^b Means followed by the same letter within a column are not significantly different by Tukey's multiple comparison test ($p < 0.05$).

Table 4. Egg size in ovary of *Trissolcus mitsukurii* emerging from different host species

Host species	Age of females					
	Just after emergence			At 7 days		
	<i>n</i>	Length (μm) ^a	Width (μm) ^a	<i>n</i>	Length (μm) ^a	Width (μm) ^a
<i>Nezara viridula</i>	400	219.2±0.8 a ^b	60.0±0.5 a	400	234.7±0.8 a	66.8±0.4 a
<i>Plautia crossota stali</i>	371	226.3±0.9 b	64.4±0.5 b	292	243.9±1.1 b	69.1±0.5 b
<i>Halyomorpha halys</i>	400	234.7±0.9 c	67.1±0.6 c	390	263.3±0.8 c	79.9±0.5 c

^a Mean±SE.^b Means followed by the same letter within a column are not significantly different by Tukey's multiple comparison test ($p<0.05$).

fecundity (number of mature eggs in the ovary just after emergence) of females emerging from *P. crossota stali* was significantly greater than that of the other species ($p<0.05$, Tukey's test). The fecundity of *T. mitsukurii* without regard to host species increased over the next 7 days. At the seven-day test, the relative fecundity assumed the following order (from least to greatest): *N. viridula*, *P. crossota stali*, *H. halys*. Differences among fecundity of females emerging from different host species were significant ($p<0.05$, Tukey's test).

The length and width of mature eggs in the ovary of *T. mitsukurii* followed the same order (from least to greatest: *N. viridula*, *P. crossota stali*, *H. halys*) without regard to the age of the female (Table 4).

Longevity of adult parasitoids

Table 5 shows the age-specific survival rates and longevity of *T. mitsukurii* emerging from the three different hosts. Females lived longer than males emerging from the same hosts. The significantly different lifespans of individuals according to hosts assumed the following order (from least to greatest): *N. viridula*, *P. crossota stali*, and *H. halys* ($p<0.05$, Tukey's test).

DISCUSSION

A positive correlation between host size and parasitoid size has been demonstrated for some species of solitary parasitoids (King, 1987). In this study we also showed that the host species affects the adult size of *T. mitsukurii* (Table 2). The number of eggs in the ovaries of females just after

Table 5. Life span of adults of *Trissolcus mitsukurii* emerging from different host species

Host species	Longevity (days) ^a	
	Male	Female
<i>Nezara viridula</i>	9.6±0.9 a ^b (33) ^c	11.0±0.5 a (135)
<i>Plautia crossota stali</i>	13.7±1.4 b (24)	25.1±1.3 b (36)
<i>Halyomorpha halys</i>	16.1±2.0 c (29)	28.5±1.6 c (49)

^a Mean±SE.^b Means followed by the same letter within a column are not significantly different by Tukey's multiple comparison test ($p<0.05$).^c Number of parasitoids used is shown in parentheses.

emergence from *P. crossota stali* was significantly greater than that of parasitoids emerging from *N. viridula* and *H. halys* (Table 3). The reason for this difference is unknown; the fecundity of females after 7 days assumed an order more closely corresponding to respective host sizes (*N. viridula*<*P. crossota stali*<*H. halys*). The size of the adult females appears to influence fitness by affecting searching efficiency, longevity, and egg supply (Godfray, 1993). The size of *T. mitsukurii* from different hosts was positively correlated to fecundity at 7 days and overall longevity (Tables 3 and 5). These results confirm the likelihood that the size of *T. mitsukurii* affects its fitness.

Another *Trissolcus* parasitoid, *T. basalis*, has been introduced to control *N. viridula* in the Pacific as a classical biological control agent (Greathead, 1986). In Brazil it was suggested that the efficacy of inoculative release could be an important factor

in the control *N. viridula* in soybean fields (Corrêa-Ferreira and Moscardi, 1996). In Japan, neither *T. basalis* nor any other egg parasitoids have yet been used to control *N. viridula*. In Hawaii, it is feared that *T. basalis* attacks non-target hosts (Follett et al., 1999). At the present it is not recommended to introduce *T. basalis* in Japan because of such non-target effects.

N. viridula is distributed in the plains of south-west Japan and attacks rice and vegetables such as legumes. *N. viridula* also invades greenhouses in autumn and damages eggplants where restrictions on the use of chemical pesticides require the use of pollinators and natural enemies in Aki City and Geisei Village, Kochi Prefecture (Arakawa, unpublished). On the other hand, *P. crossota stali* and *H. halys* are distributed throughout Japan and attack orchard trees and several kinds of beans but not rice (Yasunaga et al., 1993). These species have not been reported to invade greenhouses. According to differences in the distribution of these stink bugs, the large size *T. mitsukurii* emerging from *P. crossota stali* or *H. halys* may not attack *N. viridula* in rice fields and greenhouses.

In this study we showed the larger size *T. mitsukurii* has higher fecundity than the smaller one. *T. mitsukurii* is known as a possible controlling factor of *N. viridula* (Kiritani and Hokyo, 1962) and the larger size *T. mitsukurii* shows more possibility as a control agent of *N. viridula*. No release tests in the field using *T. mitsukurii* to control *N. viridula* have been conducted in Japan yet. The release of larger size *T. mitsukurii* in rice fields or greenhouses to control *N. viridula* can be expected in the future. For successful biological control by larger size *T. mitsukurii* knowledge of the other biological characteristics such as developmental period and competitive capacity and an established mass rearing method are necessary.

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