Development and Sex Ratio of the Parasitoid *Chrysocharis pentheus* (Hymenoptera: Eulophidae) on the Leafminer *Liriomyza trifolii* (Diptera: Agromyzidae)

Sasaki, Fumi

Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

Ueno, Takatoshi

Laboratory of Insect Natural Enemies, Division of Biological Control, Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University

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Development and Sex Ratio of the Parasitoid *Chrysocharis pentheus* (Hymenoptera: Eulophidae) on the Leafminer *Liriomyza trifolii* (Diptera: Agromyzidae)

Fumi SASAKI¹ and Takatoshi UENO*

Laboratory of Insect Natural Enemies, Division of Biological Control, Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University, Fukuoka, 812–8581, Japan

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Leafmining flies such as *Liriomyza trifolii* and *L. sativae* are serious invasive pests of many agricultural and ornamental crops. Biological control with native parasitoids can be a promising approach to manage the leafminers. The larval parasitoid *Chrysocharis pentheus* is a common and widespread native natural enemy of *Liriomyza* spp. in Asia but many aspects of its biology remain unclear. Here, the laboratory culture of *C. pentheus* with *L. trifolii* was established and its development on the host was investigated. The percentage of parasitized hosts producing the parasitoid offspring was nearly 90% at 25°C. Under low temperature/short day conditions, *C. pentheus* developed normally though the offspring survival was lower than that under 25°C conditions. Host 3rd and 2nd instars produced the parasitoid offspring with an equal proportion. However, the percentages of female parasitoids emerging from host 3rd instars were much higher than 2nd instars. Curiously, the offspring sex ratio (% females) was low at 15°C. The female offspring took longer time to finish development than males did. The offspring parasitoids were larger when emerged from host third instars than second instars. Overall, the biology of *C. pentheus* is similar to other eulophid parasitoids attacking dipteran leafminers but our results suggest that *C. pentheus* is useful as a biocontrol agent during low temperature conditions.

Key words: Biocontrol, host quality, host selection, idiobiont, IPM, sex allocation

INTRODUCTION

Leafminers in the genus of *Liriomyza* (Agromyzidae) contain several worldwide notorious pests of agricultural and ornamental crops (Minkenberg and van Lenteren, 1986; Spencer, 1989; Liu *et al.*, 2009; Foba *et al.*, 2015). However, the management of *Liriomyza* spp. is often difficult solely with synthetic insecticides because they have developed high level of resistance (Wardlow, 1985; Parrella, 1987; Weintraub and Horowitz, 1998; Ohno *et al.*, 1999a, b; Weintraub, 2001; Ueno, 2006; Matsuda and Saito, 2014; Saryazdi *et al.*, 2014; Guantai *et al.*, 2015).

In the field, *Liriomyza* spp. in the larval and pupal stages commonly suffer from a high level of parasitism by hymenopteran parasitoids (Rauf *et al.*, 2000; Gratton and Welter, 2001; Salvo *et al.*, 2005; Saito *et al.*, 2008a; Liu *et al.*, 2009; Foba *et al.*, 2015). This fact leads to an idea that biological control with such parasitoids can work well. Indeed, parasitoids have proven successful as an agent of biological control of *Liriomyza* spp. (Minkenberg and van Lenteren, 1986; Parrella *et al.*, 1987; Ozawa *et al.*, 1998, 1999, 2001; Weintraub, 2001; Chow and Heinz, 2006; Liu *et al.*, 2009).

In Japan, the community of leafminer parasitoids is rich, containing a variety of species that are native to the country (Arakaki and Kinjo, 1998; Konishi, 1998; Tokumaru and Abe, 2006; Saito *et al.*, 2008a, b; Hayashi and Kitamura, 2010). Use of the native parasitoids in biological control can thus be a promising approach to man-

age *Liriomyza* pests. Most previous studies have focused on only a few species of native parasitoids, such as *Diglyphus isaea*, *Neochrysocharis formosa* and *Hemiptarsenus varicornis* (Saito *et al.*, 1995; Arakaki and Kinjo, 1998; Konishi, 1998; Ohno *et al.*, 1999a, b; Ozawa *et al.*, 1999, 2001; Thu and Ueno, 2002; Tran *et al.*, 2007; Ho and Ueno, 2008; Tran and Ueno, 2012; Tran *et al.*, 2012). However, there are still several unfocused native species that may be good biocontrol agents.

Chrysocharis pentheus is a common parasitoid attacking *Liriomyza* spp. and is widely distributed in the Palearctic Region including many Asian countries like Japan, Korea, China, and Malaysia (Konishi, 1998; Hayashi and Kitamura, 2010; Chien and Chang, 2013). This parasitoid is abundant in early spring and late autumn when other major leafminer parasitoids are rather scarce (Iguchi, 1997; Mitsuda and Yamasaki, 2003; Hayashi and Kitamura, 2010). Hondo *et al.* (2006) have also suggested that *C. pentheus* is adapted to low temperature conditions. Thus, *C. pentheus* may be a useful agent during the period of low temperature conditions. However, very few studies so far have assessed the importance of *C. pentheus* in suppressing leafminer populations and its usefulness as a biological control agent. To evaluate the potential of a certain parasitoid species as a biocontrol agent, it is essential to study its biological characteristics such as development time, adult longevity, fecundity, host range, sex ratio, host-finding capacity, and host selection behavior (van Driesche and Bellows, Jr., 1996; Jervis, 2005). Such basic information is limited for *C. pentheus*, however.

The objectives of the present study are to investigate biological parameters of the parasitoid. Specifically,

¹ Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, Japan

* Corresponding author (E-mail: ueno@grt.kyushu-u.ac.jp)

experiments were conducted to examine the development and offspring sex ratio of *C. pentheus* under low and high temperature conditions. Basing on the results, we will discuss the potential and usefulness of *C. pentheus* in leafminer management.

MATERIALS AND METHODS

Host and parasitoids

Seeds of kidney bean were singly planted in plastic pots (5.5 cm in diameter). After around one week of germination, a shallow pan (32 cm*44 cm*6 cm) containing 24 pots with kidney bean plants was placed on a large shelf covered with a fine meshed nylon. Leafminer adults were released into the shelf for 24 h. After a 24 h exposure for oviposition, the potted plants were transferred on a different shelf maintained at $25 \pm 1^\circ\text{C}$ and 16L:8D. The plants were kept until most leafminer larvae were matured.

Chrysoscharis pentheus used in our study was originated from individuals that had emerged from tomato leaves with *L. trifolii* at the campus of Kyushu University. The culture of *C. pentheus* was maintained on larvae of *L. trifolii*. Six potted kidney bean plants infested with 3–6 third instars of *L. trifolii* were introduced into a transparent plastic box (32 cm*17 cm*25 cm), the upper side of which was covered with a fine meshed nylon. Mated female parasitoids were then introduced into the box. Tissue paper saturated with honey solution was also placed in the box as a food source for the female parasitoids. Female parasitoids were allowed to parasitize leafminer larvae for 24 hours. After 24 hours for parasitism, plants with parasitized hosts were removed from the box, and were kept in the rearing room ($25 \pm 1^\circ\text{C}$, 24 h light) for 2–6 days. Leaves containing parasitized larvae were then cut off from the plants, and were singly placed in a glass schale (9 cm in diameter) for parasitoid emergence.

Experimental procedures

A leaf of kidney bean infested with 3–6 host second and third instars was placed in a glass schale (9 cm in diameter). One female *C. pentheus* of 3 days old was introduced into it, and was kept for 24 h in an incubator maintained at $25 \pm 0.5^\circ\text{C}$, 16L:8D. Then, kidney bean leaves were removed from the schale and were observed under a binocular microscope to check whether leafminers had been parasitized. Host paralysis was used to determine parasitism by *C. pentheus*. Because *C. pentheus* is a destructive host-feeding parasitoid, hosts used for host-feeding die without producing any parasitoid offspring. To evaluate the exact host suitability for development of *C. pentheus*, we carefully examined whether host larvae had been fed on by female *C. pentheus* or not, and those that had appeared to be used for feeding were discarded. Leaves with parasitized hosts were held in incubators that were maintained at $25 \pm 0.5^\circ\text{C}$ under 16L:8D or $15 \pm 0.5^\circ\text{C}$ under 11L:13D. Parasitized hosts were observed on the daily basis to check the development of the offspring parasitoids. When adult parasitoids were eclosed, the day and the sex of

the parasitoids were recorded. The head width of newly eclosed parasitoids was measured under a binocular microscope, as an index of the size.

The data were analyzed with JMP (2010). The distributions of development time and wasp size were fitted to normal distributions before the statistical treatments, and parametric procedures were then applied to analyze statistical differences. The offspring survival and sex ratio were analyzed with multiple logistic regression analyses in which temperature and host instar were included as independent variables.

RESULTS

A laboratory culture of *C. pentheus* was successfully established using *L. trifolii* as a host. The parasitoid was easily reared in the laboratory using the rearing procedure that was basically the same with *H. varicornis* – *L. trifolii* system (Thu and Ueno, 2002). The following experiments were undertaken with laboratory-reared parasitoids.

In all, 346 host larvae were parasitized by female *C. pentheus* in the experiment, and 147 and 199 parasitized larvae were reared at 15°C and 25°C , respectively. Laboratory rearing demonstrated that *C. pentheus* developed successfully on larvae of *L. trifolii*. A multiple logistic regression analysis showed that temperatures significantly affected the likelihood of successful development to adulthood whereas host instars (2nd versus 3rd larval instars of *L. trifolii*) did not (Table 1). The percentages of successful development, measured as the percentages of hosts that had produced parasitoid adults, were 63.9% and 89.4% at 15°C and 25°C , respectively, when data were combined for host instars (Figure 1).

The mortality of developing parasitoids mostly occurred at larval stages, and 34.7% of individuals died before pupation at 15°C whereas only 3.0% did so at 25°C . The difference was highly significant (chi-squared test; $\chi^2 = 66.0$, $P < 0.0001$). The mortality during the pupal stage was low (3.4% at 15°C and 7.5% at 25°C) and did not significantly differ between the temperature groups (chi-squared test; $\chi^2 = 2.81$, $P = 0.094$).

The mean development times from egg to adult eclosion were summarized in Table 2. Temperature had a strong influence to development, and wasps eclosed around 2 weeks after parasitization at 25°C and 1 month at 15°C . Statistical results based on a three-way ANOVA showed that host instars did not affect developmental time whereas wasp sex and temperature had significant

Table 1. A multiple logistic regression analysis to assess the effect of temperature and host instar on the survival of parasitoid offspring

Factors	χ^2 values	P values
Temperature	32.5	<0.0001
Host instar	0.49	0.48

Whole model was highly significant ($P < 0.0001$). Interaction between the two factors was not significant.

Table 2. Developmental time in days of *Chrysocharis pentheus* reared on *Liriomyza trifolii*

Temperature	Sex	Total development time	Egg-larval period	Pupal period
15	Female	30.8±2.9	15.5±2.7	15.4±1.8
	Male	29.7±3.1	15.2±2.8	14.5±1.5
25	Female	16.0±1.4	8.2±1.3	7.8±0.9
	Male	14.6±1.3	8.0±2.1	6.6±1.6

See also Table 3 for the statistical results.

Fig. 1. Effect of temperature (15°C versus 25°C) on the survival of offspring *C. pentheus* (= proportion of hosts producing parasitoid offspring). Different letters above bars show a significant difference between the groups (chi-squared test; $P < 0.0001$). See also Table 1 for the statistical result.

Fig. 2. Offspring sex ratio at the time of wasp emergence in relation to host instars at two different rearing conditions (above; 15°C, below; 25°C). Different letters above bars show a significant difference between the groups (chi-squared test; $P < 0.05$). See also Table 4 for the statistical result.

Table 3. The results of three-way ANOVA for factors affecting development time of *C. pentheus* (from egg to adult eclosion)

Factors	<i>t</i> values	<i>P</i> values
<i>Total development time</i>		
Temperature	54.08	< 0.0001
Sex	3.85	0.0001
Host instar	-0.75	0.45
<i>Egg-larval period</i>		
Temperature	24.85	< 0.0001
Sex	0.41	0.69
Host instar	-0.53	0.60
<i>Pupal period</i>		
Temperature	40.73	< 0.0001
Sex	5.00	< 0.0001
Host instar	-0.27	0.78

Whole models were highly significant ($P < 0.0001$). Interactions were not significant and were hence excluded from the analyses.

Table 4. The result of a multiple logistic regression analysis for wasp sexes emerging from second versus third host instars at two different temperature conditions

Factors	χ^2 values	<i>P</i> values
Temperature	13.50	0.0002
Host instar	36.33	< 0.0001

Whole model was highly significant ($P < 0.0001$). Interaction was not significant and was hence excluded from the analysis.

Table 5. The result of a two-way ANOVA for wasp size (head width) in relation to wasp sex and host instars

Factors	<i>t</i> values	<i>P</i> values
Wasp sex	4.21	< 0.0001
Host instar	-2.85	0.0056
Development time	-1.55	0.126

Whole model was highly significant ($P < 0.0001$). Interaction was not significant and was hence excluded from the analysis.

Fig. 3. The size of *C. pentheus* emerging in relation to wasp sex. Different letters above bars show a significant difference between the groups (one-way ANOVA; $P < 0.0001$). For data from second instars, the size of females is not shown because only one female individual emerged from host 2nd instar. See also Table 5 for the statistical result.

impacts (Table 3). Males developed significantly but slightly faster than females did (Tables 2). Curiously, development time during the larval stage did not differ between the sexes but the sexual difference in time appeared during the pupal stage (Table 3).

The offspring sex ratio at the time of wasp eclosion differed depending on host instars and temperatures (Table 4). The third instars of *L. trifolii* produced significantly more female parasitoids than the second instars did, regardless of temperature conditions (Figure 2). However, the proportion of female *C. pentheus* produced was much lower at 15°C than 25°C (Figure 2).

The size of *C. pentheus* was measured for 91 individuals reared at 15°C. The head width as an index of wasp size was significantly affected by wasp sex and host instars (Table 5). Basically, wasp size was larger in females than males (Figure 3). Host third instars produced larger *C. pentheus* than second instars did though comparison was not possible for females because only one individual was produced from second host instar (Figure 3).

DISCUSSION

The present study shows that *Chrysocharis pentheus* can easily be maintained in the laboratory with rearing procedures used for other eulophid parasitoids of dipteran leafminers such as *Hemiptarsenus varicornis*, *Diglyphus isaea* and *Neochrysocharis formosa* (e.g., Thu and Ueno, 2002; Tran *et al.*, 2007). When *L. trifolii* was used as a host of *C. pentheus* at 25°C, the percentage of hosts successfully producing parasitoid offspring was nearly 90%, indicating that *L. trifolii* is a suitable host for *C. pentheus*. This might be somewhat curious because the leafminer *L. trifolii* is an exotic species while *C. pentheus* is native to Japan. *Chrysocharis pentheus* has been recorded from a variety of dipteran leafminers belonging to the genera *Liriomyza*,

Phytomyza and *Chromatomyia* (e.g., Sugimoto and Ishii, 1979; Sugimoto *et al.*, 1982; Iguchi, 1997; Konishi, 1998; Tokumaru and Abe, 2006; Saito *et al.*, 2008a, b). In addition, this parasitoid is able to use a lepidopteran leafminer, *Phyllocnistis citrella* (the citrus leafminer) as host (Mafi and Ohbayashi, 2004). Hence, the polyphagous nature will enable *C. pentheus* to parasitize and develop on a wide range of different leafmining hosts. This parasitoid thus should have a potential to use exotic *Liriomyza* species as host.

At a low temperature condition of 15°C, the survival of *C. pentheus* was reduced to around 60%. Mortality during the larval stage caused the reduced survival. Although *C. pentheus* is rather cold tolerant (Hondo *et al.*, 2006), the likelihood of larval mortality may increase under low temperatures. Alternatively, larval survival is reduced because the quality of leaf containing parasitized leafminers is lowered during a relatively long period of rearing (around one month in our study) and because leaf of lowered quality does not provide a suitable micro-environment for the developing *C. pentheus* within the mine of host.

It is noticeable that *C. pentheus* develops to adult even under the low temperature/short day condition of 15°C/11L:13D. With this condition, insects of the temperate region would undergo diapause to survive winter (Tauber *et al.*, 1986). It is unknown what development stage *C. pentheus* undergo diapause. However, the present result does suggest that adult *C. pentheus* occur in late autumn and early spring. Previous studies in fact have demonstrated that high levels of parasitism by *C. pentheus* are detected in late autumn and early spring when other leafminer parasitoids are rather scarce (Iguchi, 1997; Mitsuda and Yamasaki, 2003).

Neochrysocharis formosa, which is also a leafminer eulophid parasitoid with a southern distribution, requires 1.5 months from oviposition to adult emergence (Hondo *et al.*, 2006) while *C. pentheus* finished the development around one month (Table 2). Relatively fast development at 15°C in *C. pentheus* suggests that this parasitoid is adapted to cool environments. Indeed, Hondo *et al.* (2006), who compared thermal tolerance of eight native parasitoids of dipteran leafminers, demonstrated that *C. pentheus* is adapted to low temperatures. Curiously, *C. pubicornis*, a related species of *C. pentheus*, undergoes summer diapause; the adults terminate the diapause and emerge under low temperature conditions (Baeza Larios *et al.*, 2007). *Chrysocharis pentheus* may be useful for biological control during growing seasons between autumn and early spring.

In general, host size or instar affects the survival of developing parasitoids; parasitoid survival is mostly lower in smaller hosts or younger host instars (Vinson and Iwantsch, 1980; Godfray, 1994; Ueno, 1999). In the present study, we did not detect such an effect at least between host second and third instars. The host *L. trifolii* appears suitable for development of *C. pentheus*, regardless of the host stage.

Sexual difference in size is common among parasitoids including leafminer eulophids, and a consistent pat-

tern is that females are larger than males (Bordat *et al.*, 1995; Ode and Heinz, 2002; Bazzocchi *et al.*, 2003). This was the case for *C. pentheus* in the present study. In general, larger host leafminers produce larger eulophid parasitoids (Croft and Copland, 1995; Ode and Heinz, 2002; Salvo and Valladares, 2002). Because female eggs are more likely to be laid on larger hosts in eulophids (Heinz and Parrella, 1990; Ode and Heinz, 2002), sexual difference in size should result, at least in part, from a difference in host size used for male versus female offspring.

For most of parasitoids examined so far, the development time of males is shorter than that of females (Ueno and Tanaka, 1994; Quicke, 1997). Similarly, in *C. pentheus*, males developed faster than females. This sexual difference arose during the pupal stage, not during the larval stage. It is not known why such a sexual difference emerges during the pupal stage.

The sex ratio of parasitoid wasps is variable in response to host-associated factors such as host size, age, stage and prior parasitism, and host size is one of the major factors influencing parasitoid sex ratio (King, 1987; Godfray, 1994; Ueno, 1999; Ode and Heinz, 2002; Ueno, 2015). In the present study, host third instars yielded more female *C. pentheus* than second instars. Because the former host larvae are much larger than the latter, the difference in size between the two host instars is considered responsible for the difference in observed sex ratio. Similar results are obtained for other leafminer parasitoids, and host-size dependent sex ratio may be a general rule for leafminer parasitoids (Croft and Copland, 1995; Ode and Heinz, 2002; Chow and Heinz, 2005, 2006).

In addition, physical factors such as temperature and humidity may influence parasitoid sex ratio (King, 1987). In *C. pentheus*, temperatures affected the offspring sex ratio. Production of fewer female offspring under lower temperatures has not been recorded for leafminer parasitoids so far. How low temperatures affect the parasitoid sex ratio is not clear. A likely explanation is that the survival of immature females is lower at lower temperatures. In fact, the overall survival of *C. pentheus* was lower at 15°C than 25°C. Sex-dependent mortality in response to temperature is not confirmed in any parasitoids, but this should be examined in the future study.

Curiously, the sex ratio of *C. pentheus* is biased toward males. Male-biased sex ratio is often reported for a number of parasitoid wasps including leafminer parasitoids used for biological control (Ode and Heinz, 2002; Chow and Heinz 2005, 2006). It is not known why the sex ratio of *C. pentheus* is male-biased. Because male-biased sex ratio results in low efficiency of mass production of the parasitoid, the future study also should address this question to improve the sex ratio.

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