Biology of *Chrysocharis pentheus*, an Endoparasitoid Wasp of the Citrus Leafminer *Phyllocnistis citrella* Stainton

Sh. Mafi¹*, and N. Ohbayashi²

ABSTRACT

The biology of *Chrysocharis pentheus* (Walker) (Hymenoptera: Eulophidae), the most abundant endoparasitoid of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in Ehime prefecture, Japan, was studied under laboratory and field conditions. The mean immature developmental time and adult longevity at different temperatures (22 to 31°C) under 50-70% RH decreased as the temperature increased, and females survived longer than males. The developmental threshold of male and female was 8.9°C and 11.9°C, respectively. The effective accumulative temperature (thermal constant) for males and females from egg to adult was 181.8 and 238.1 degree-days, respectively.

*C. pentheus* appeared to be a synovigenic species. The offspring sex ratio from females provided with males was 73.8% males and 26.2% females. Presumably, mated females began oviposition 1-2 days after emergence and continued for up to 40 days. Females laid a mean of 118.2±10.10 eggs each, and their longevity reached 32.3±0.30 days at 27±1°C, 50-70% RH. The intrinsic rate of natural increase (rm) was 0.144. Host feeding or stinging without oviposition killed 65.7±8.65 *P. citrella* larvae per female. Oviposition in the field was highest (62.7%) on the third instar larva of *P. citrella*, with the rest on the prepupal stage (37.3%), showing a significant preference for third instars. The sex ratio (M:F) of *C. pentheus* reared from the third instar larvae of host and pupae was 3.3:1.0 and 1.0:3.0 respectively.

Keywords: *Chrysocharis pentheus*, Fecundity, Longevity, *Phyllocnistis citrella*, Sex ratio.

INTRODUCTION

The citrus leafminer, *Phyllocnistis citrella* Stainton (CLM), is an important pest of citrus and related Rutaceae throughout almost all the world (Clausen, 1931; Badawy, 1967; Heppner, 1993). The development of resistance of CLM to commercially available insecticides in some parts of Japan has led to a search for alternative methods such as biological control or an IPM program (Ujiye, 2000). Biological control plays an important role in reducing leafminer densities in Australia (Smith and Beattie, 1996), Thailand (Morakote and Nanta, 1996), and Florida groves (Hoy *et al.*, 1997; Xiao *et al.*, 2007).

There is a diverse complex of hymenopterous parasitoids attacking *P. citrella* (Pena *et al.*, 1996; Schauff *et al.*, 1998), mostly eulophids, but also some encyrtids, elasmids, eurytomids, eupelmids, and peteromalids (Ishii, 1953; Ujiye *et al.*, 1996). More than 32 species of chalcidoids were identified in Japan, Taiwan and Thailand, including *Chrysocharis pentheus* (Walker) (Hymenoptera: Eulophidae) which was the dominant species in Wakayama prefecture of Japan (Ujiye and Adachi, 1995). High levels of parasitism (70%) in the Matsuyama area were caused by seven species of eulophidiae, among each *C. pentheus* was the most abundant species (Mafi and Ohbayashi, 2004). *Chrysocharis pentheus* is an endoparasite and

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So far, hymenopterous parasitoids have proven to be a successful biological control agent on several insect pests. But to be used as biological control agents, parasitoids must be carefully studied and their biology well understood. Since the biology of *C. pentheus* is not fully understood, in this paper we investigated biological parameters including development time at different temperatures, fecundity, sex ratio, adult longevity and host preference of *C. pentheus* on its host *P. citrella*.

**MATERIALS AND METHODS**

The developmental stages of *P. citrella* and its parasitoid wasp, *C. pentheus* used in this study were collected from the citrus orchards of Ehime Fruit Tree Experiment Station (33.50 N, 132.42 E, elevation 32 m). The CLM populations were also maintained on two year-old *Citrus iyo* trees in a greenhouse following a methodology similar to that described by Smith and Hoy (1995). The adult wasps used in these studies were collected at the pupal stage and reared in Petri-dishes (9×2.5 cm) with 70% sugar water. They were kept at 25°C under 50-70% RH in an incubator for the different studies (e. g. longevity of adult parasitoids).

**Developmental Time at Different Temperatures**

The developmental time from egg to adult of *C. pentheus* was investigated at 22, 25, 28, and 31°C, 50-70% RH, and 12 L:12 D photoperiod. Female wasps of various ages were randomly collected by aspirators from third instar larvae (5-10 larvae) were placed in Petri-dishes (9×2.5 cm). To keep the leaves fresh, wet paper was attached to the leaf petioles. Two or three female wasps were introduced into each Petri-dish and allowed to oviposit for a day. Females were then removed from Petri-dishes and parasitized hosts were kept in an incubator at different temperatures. The development of the parasitoid was observed daily until adult emergence. The developmental threshold $T_0$ and the thermal constant ($K$) were determined based on the total developmental time from egg to adult at each temperature.

**Adult Longevity**

The longevity of adult parasitoids was investigated under laboratory conditions at 22, 25, 28, and 31°C, 50-70% RH, 12 L:12 D. Newly emerged adults were individually placed in plastic Petri-dishes (9×2.5 cm) with droplets of 70% sugar water as food source. Parasitoid mortality was observed daily.

**Sex Ratio**

The immature stages of the parasitoid in the CLM larvae and pupae were collected from the field monthly from June to October and reared to adult as above. The Petri-dishes were kept at 25±1°C and 12 L:12 D photoperiod in an incubator. The sex ratio of emerged adult parasitoids was determined separately by host stage.

**Host Stage Preference and Superparasitism**

To determine the preference of host stage and assess superparasitism, all the developmental stages of CLM larvae and pupae (n= 200 per each stage) were randomly collected from field. The samples...
were examined under a stereomicroscope to determine the presence of parasitoid eggs or larvae inside the host body. The preference of host instar larvae was determined by counting the number of molted head capsules in the mine.

Reproduction

Pre-oviposition, post-oviposition, and oviposition periods, fecundity and host mortality were determined by daily observations of individual female parasitoids at 27±1°C, 50-70% RH, and 12 L:12 D. Adult females (n= 10) offered with second and third instars (n= 15) P. citrella on detached leaves. Leaves were placed in a transparent plastic box (3×11×13 cm) with nylon mesh on one side. One recently emerged female less than 24 hours old and one male parasitoid were introduced into each box. The parasitoids were fed with small drops of 70% sugar water provided on the leaves. The fate of the exposed larvae and the parasitoids was recorded daily and a new set of host larvae re-introduced into the box. The larvae were checked under a stereo-microscope for evidence of either host feeding (dead leafminer larvae exhibiting many black sting marks) or oviposition (observation of parasitoid larvae inside the host body). Parasitized larvae were transferred to another plastic box and kept in the incubator until adult parasitoid emergence. Female parasitoids were maintained for their entire life and males were replaced as they died. The intrinsic rate of natural increase (r\textsubscript{m}), net reproductive rate (R\textsubscript{0}) and mean generation time (T) were computed according to the equations given by Birch (1948).

Data Analysis

To determine the effect of various temperatures on the developmental time and longevity of C. pentheus, a one-way ANOVA and Tukey’s test (α= 0.01) was performed. Other data were analyzed with a chi-squared test using JMP (SAS Institute, 2001).

RESULTS

Developmental Time at Different Temperatures

The developmental time decreased for all stages as the temperature increased (Table 1). Developmental time from 22 to 31°C ranged from 16.9 to 9.2 days and 17.7 to 10.6 days for males and females, respectively. A significant difference was found among developmental time between the sexes at various temperatures (for males: ANOVA, P< 0.01, F= 167.05 df= 57, CV= 7.84 and for females: ANOVA, P< 0.01, F= 73.79 df= 24, CV= 7.87). An ANOVA analysis did not reveal any significant difference of developmental time between

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Days from egg to pupa (Mean±SE)</th>
<th>Days from pupa to adult (Mean±SE)</th>
<th>Days from egg to adult (Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>22</td>
<td>7.9±0.47(32)</td>
<td>9.2±0.92(32)</td>
<td>16.9±1.12(20)a</td>
</tr>
<tr>
<td>25</td>
<td>6.5±0.52(34)</td>
<td>7.6±0.68(34)</td>
<td>14.0±0.77(20)b</td>
</tr>
<tr>
<td>28</td>
<td>5.7±0.71(30)</td>
<td>6.1±0.68(30)</td>
<td>11.6±0.89(20)c</td>
</tr>
<tr>
<td>31</td>
<td>4.8±0.74(33)</td>
<td>5.6±1.11(33)</td>
<td>9.2±0.51(20)d</td>
</tr>
</tbody>
</table>

The numbers in parentheses are the actual number of parasitoid wasp developmental stages. Means followed by different letters in the same column are significantly different in one-way ANOVA and Tukey’s test P< 0.01.
Table 2. Estimated developmental threshold and thermal constants from egg to adult emergence for C. pentheus.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Regression equation (^a)</th>
<th>(R^2)</th>
<th>(p)</th>
<th>Developmental threshold (T_0) (°C)</th>
<th>Thermal constant (K) (degree-days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>(V=0.0055T-0.0645)</td>
<td>0.9774</td>
<td>&lt;0.001</td>
<td>11.9</td>
<td>181.8</td>
</tr>
<tr>
<td>Female</td>
<td>(V=0.0042T-0.0374)</td>
<td>0.9996</td>
<td>&lt;0.001</td>
<td>8.9</td>
<td>238.1</td>
</tr>
</tbody>
</table>

\(^a\) V: Developmental velocity, T: Temperature.

Sexes at each temperature (P> 0.05), but males developed slightly faster than females at all temperatures tested. The relationship between temperature and the developmental rate, developmental threshold and thermal constant of males and females are summarized in Table 2. The developmental threshold of males and females was 8.9°C and 11.9°C, respectively, and the thermal constant for males and females from egg to adult was 181.8 and 238.1 degree-days, respectively.

Adult Longevity

On sugar water (70%), the longevity of female and male parasitoids increased as the temperature decreased (Table 3). A significant difference was detected for parasitoid longevity at various temperatures. Females survived longer than males at all the temperatures tested. The greatest longevity for females and males was 56 and 52 days at 22°C, respectively. Adult wasps fed with only water or without food died within a few days (unpublished).

Sex Ratio of Parasitoids

Throughout the season, 823 adult parasitoids were reared from the susceptible leafminer stages, i.e. third instar larvae and pupae. Parasitoids that emerged from third instar larvae were predominantly males (76.8%), and those from pupae were mostly females (75.6%) (Table 4). An \(\chi^2\) test of independence revealed a dependent relationship (\(\chi^2 = 26.8, df= 1, P< 0.0001\)) between the host stage and parasitoid sex every month. The relationship was independent for host stages during the different months (\(\chi^2 = 4.63, df= 4, P= 0.3280; \chi^2 = 2.17, df= 4, P= 0.706\)), respectively. The sex ratio (M:F) of emerged parasitoids from third instar and pupae was 3.3:1.0 and 1.0:3.0, respectively.

Table 3. The effect of temperature on longevity of adult C. pentheus, provided with 70% sugar water.

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Female Average longevity (days±SE)</th>
<th>Male Average longevity (days±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>33.9±6.17 (41)a</td>
<td>28.6±7.55 (37)a</td>
</tr>
<tr>
<td>25</td>
<td>27.8±8.79 (28)b</td>
<td>23.7±7.05 (27)b</td>
</tr>
<tr>
<td>28</td>
<td>17.6±3.66 (27)c</td>
<td>14.4±3.91 (32)c</td>
</tr>
<tr>
<td>31</td>
<td>16.4±2.78 (19)c</td>
<td>12.8±3.43 (23)c</td>
</tr>
</tbody>
</table>

The numbers in parentheses indicate sample size. Means followed by different letters in the same column are significantly different in one-way ANOVA and Tukey’s test P< 0.01.
Table 4. Sex ratio of *C. pentheus* emerged from different stage of *Phyllocnistis citrella*.

<table>
<thead>
<tr>
<th></th>
<th>From the third instar larva of CLM</th>
<th>From the pupa of CLM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>Male : Female</td>
<td>Male : Female</td>
</tr>
<tr>
<td>June</td>
<td>78% (47) : 22% (13)</td>
<td>22% (10) : 78% (35)</td>
</tr>
<tr>
<td>July</td>
<td>82% (115) : 18% (25)</td>
<td>29% (35) : 71% (85)</td>
</tr>
<tr>
<td>August</td>
<td>74% (52) : 26% (18)</td>
<td>23% (13) : 77% (43)</td>
</tr>
<tr>
<td>September</td>
<td>80% (41) : 20% (11)</td>
<td>19% (4) : 81% (18)</td>
</tr>
<tr>
<td>October</td>
<td>70% (68) : 30% (28)</td>
<td>29% (23) : 71% (57)</td>
</tr>
<tr>
<td>Average</td>
<td>76.8±4.8% : 23.2±4.8%</td>
<td>24.4±4.4% : 75.6±4.4%</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>3.3 (323) : 1.0 (92)</td>
<td>1.0 (85) : 3.0 (238)</td>
</tr>
</tbody>
</table>

*a* Number in parentheses means individual number of *Chrysocharis pentheus*. Average showed by Mean±SD.

**Host Stage Preferences and Superparasitism**

More eggs of *Chrysocharis pentheus* were found in third instar larvae (62.7%) than in prepupae (37.3%). No egg oviposition occurred at the first and second instar or pupal stage of CLM under field and laboratory conditions. Thus, females preferentially oviposit on third instar larvae than prepupae ($\chi^2 = 239.5$, df= 4, P< 0.0001). Superparasitism was relatively rare under both laboratory as well as field conditions.

**Reproduction**

*Chrysocharis pentheus* appeared to be a synovigenic species which matured eggs throughout its adult life. The presumably mated females began oviposition 1-2 days after emergence and continued up to day 40 (Figure 1). Daily reproduction was dependent on female age, gradually increasing until day 6. Oviposition generally declined subsequently with several fluctuations and was reduced to zero by day 40. The peak of age-specific fecundity of 7.3±1.1 eggs/female per day was observed on five day-old females. Individual fecundity varied from 71 to 187 with a mean number of 118.2±10.1 progeny. Fertility was quite high (92.0%) with an average offspring sex ratio of 73.8% male and 26.2% female. The intrinsic rate of natural increase ($r_m$) was 0.144. The net reproductive rate ($R_0$) and mean generation time (T) were 53.85 and 12.03, respectively. The post-oviposition period was very long (5.0±0.74 day) when compared with the pre-oviposition period (0.6±0.22 day). Host larval mortality from oviposition was 118.2±10.1 per female, ranging from 71 to 187.

Host killing by adult females without parasitism began simultaneously with oviposition behavior and ended shortly after the parasitoids ceased ovipositing. Female parasitoids exhibited their highest host-killing peak approximately 19 days after emergence, killing 2.8±1.8 host larvae/female per day (Figure 1). The number of hosts killed by parasitoid feeding or stinging without oviposition reached 65.7±8.65 per female. Approximately, 35.52% of host larval mortality was caused by feeding or the stinging activity of adult females.

**DISCUSSION**

Parasitoid wasps can be divided into two major groups in terms of patterns of egg production: “pro-ovigenic” and “synovigenic” parasitoids (Flanders, 1950).
The present observations indicate that *C. pentheus* is a typically synovigenic parasitoid, the females producing eggs throughout their lifetime (Figure 1). In synovigenic parasitoids, adult nutrition is known to influence longevity and egg production during the lifetime (Leius, 1961a, b; 1962; 1967). Many synovigenic parasitoids use their host insects either as oviposition sites or as food sources, a behavior known as “host-feeding” (DeBach, 1943; Jervis and Kidd, 1986). By feeding upon a host, their longevity and fecundity can be increased and also host searching efficiency can be enhanced (Syme, 1977). However, there are examples where host-feeding had no effect on female longevity (Ueno and Tanaka, 1994). In the chalcidoid genus *Aphytis*, the effect of host-feeding on lifespan depends on whether sugar is included in the diet or not. In the absence of sugar, host-feeding has almost no effect on lifespan while, in the presence of sugar, host-feeding substantially increases lifespan (Collier, 1995a). In the current study the longevity of parasitoid females was increased remarkably when fed by sugar water compared with females deprived of sugar water (unpublished). The sugar water influenced fecundity and other related life history parameters. It appears that only host meal is not sufficient to sustain the survival of *C. pentheus* under laboratory and field conditions.

The developmental time of *C. pentheus* on *Phytomyza ranunculi*, decreased as the temperature increased from 15 to 32°C. The optimal temperature zone for longevity of adult females was around 20°C (Sugimoto et al., 1981). Data from the present study are in agreement with the previous reports on the subject. However, developmental time of *C. pentheus* was rather shorter on *P. citrella* compared to *P. ranunculi*. The developmental threshold of *C. pentheus* on *P. ranunculi* was higher than that calculated for *P. citrella* (10.0°C versus 7.6°C). Conversely, the thermal constant of *C. pentheus* on *P. ranunculi* was quite a bit higher than that for *P. citrella* (273.7 DD versus 212.8 DD).

Life history parameters of *C. pentheus* on *P. citrella* have previously been reported (Sugimoto and Ishii, 1979). Mean longevity and fecundity of *C. pentheus* were determined at 15.2 days and 47.3 eggs, respectively. The mean values appeared to be smaller than those in the current studies.
(32.3 days and 118.6 eggs, respectively). Conversely, the mean value of host mortality (83.2 larvae) on P. ranunculi was higher than on P. citrella (65.7 larvae). These differences can be related to the host insects and experimental conditions.

Parasitoids tend to prefer different stages of hosts for parasitization or host-feeding (DeBach, 1943; Bartlett, 1964). Sugimoto and Ishii (1979) reported that C. pentheus evidently preferred third instar larvae of P. ranunculi for oviposition and feeding, though host-feeding was recorded on second instar larvae at low preference. Our study results revealed that C. pentheus conspicuously preferred third instar larvae and prepupae of P. citrella for oviposition, whereas it evidently preferred second and especially third instar larvae for host-feeding. The preference of C. pentheus for second and third instars of P. citrella for host feeding may be economically important, since a large portion of the damage and reduction of leaf area is caused by the third instar larva of CLM (Schaffer et al., 1997).

Sex allocation behavior is common among solitary ectoparasitoids and offspring sex ratios are negatively correlated with host size (Holdaway and Smith, 1932; Seyrig, 1935; Taylor, 1937; Askew and Shaw, 1979; Barrett and Brunner, 1990; Ueno and Tanaka, 1997). This correlation is explained by a theoretical model called “host size” (Charnov, 1979; Charnov et al., 1981; Sandlan, 1979). This is because host size is correlated positively with adult wasp size through increasing amounts of host resources which has a greater effect on the fitness of females than that of males, and female offspring are expected to be allocated to larger hosts. The results of these studies demonstrated that female parasitoids tend to allocate more female offspring to pupae of P. citrella and males to the larval stage. Additional research is required to determine how the host size was estimated by the female wasps across the same larval instar or prepupa for oviposition of different sex eggs.

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Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae) - مرکبات

ش. مافی و ن. اوبایاشی

چکیده

پارازیتوپیدهای داخلی شب برمه مینوز برگ مرکبات در استان اهیم زاین، در شرایط مزرعه و آزمایشگاهی مورد بررسی قرار گرفت. متوسط طول دوره رشد مرحله ناپایه و طول عمر حشرات بالغ (21 الی 31 درجه سانتیگراد و رطوبت نسبی 50-70 درصد) با افزایش درجه حرارت کاهش یافته و طول عمر حشرات ماده طولانی تر از حشرات نر محاسبه گردید. صفر رشد

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حرارت نر و ماده به ترتیب 8/9 و 11/9 درجه سانتی‌گراد و ثابت حرارتی (Developmental threshold) از مرحله تخم تا حرارت کامل 188/1 و 188/1 درجه-روز تعیین گردید. درصد جنسی (Thermal constant) نوزادهای پروسه پایه در شرایط آزمایشگاهی 23/8 درصد نر و 24/2 درصد ماده بودند. تخم‌بری در حشرات ماده یک باد در روز بعد از ظهور شروع و تا 100 روز ادعا داشت. متوسط تعداد تخم گذاشته شده توسط یک حشره ماده در طول 23/8 ± 11 درجه سانتی‌گراد و رطوبت نسبی 50-70 درصد) با تخم ذخیره افراش 444 محاسبه گردید. متوسط مرنگ و می‌لارو (Host-feeding) میزان به روش تغذیه ای (Stinging) به ازای هر زنبور ماده 10 ± 4/7 عدد معین گردید. ترجیح میزانی توسط زنبور ماده در شرایط مزرعه ای، 6/7 درصد تخم روي سن سه لاروی میزان و 1/3 درصد روي مرحله پيش شخيرگي گذاشته شد. نسبت جنسی (M:F) زنبورهای پروسه پایه از سن سه لاروی و شخيرگي در شرایط مزرعه ای به ترتیب 1/10 و 33/3/70 تعیین گردید.