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Some biological parameters of *Sympiesis striatipes* (Hym.: Eulophidae), an ectoparasitoid of the citrus leafminer *Phyllocnistis citrella* (Lep.: Gracillariidae)

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Abstract

The biological parameters of *Sympiesis striatipes* Ashmead, one of the most abundant hymenopterous ectoparasitoid of *Phyllocnistis citrella* Stainton in Ehime province of Japan, were studied under laboratory and field conditions. The mean immature developmental time and adult longevity at different temperatures (22 to 31°C), 50-70% RH and 12L: 12D photoperiod decreased as the temperature increased, and females survived longer than males. Offspring sex ratio from females provided with males was 84.7% males and 15.3% females. Presumably mated females began oviposition 2-3 days after emergence and continued up to 39 days. Each female laid a mean of 123.4 ± 13.97, and longevity reached 33.8 ± 1.5 days at 27 ± 1°C, 50-70% RH and 12L: 12D photoperiod. The intrinsic rate of natural increase (r_m) was 0.312. Host feeding or stinging without oviposition killed 44.7 ± 4.2 host larvae per female parasitoid. Seventy five point eight percent of eggs were laid on third instar larva of host and the remainder on the prepupa. Under field conditions, superparasitism on the third instar larvae was 9.2% (n = 200) and up to 7 eggs per host were recorded. Under superparasitism lethal competition (n = 40), one adult parasitoid per host emerged from 87.5% of the samples and two adult parasitoids per host emerged from the rest. Superparasitism on prepupa was rare. The sex ratio (M: F) of the parasitoid oviposited on the third instar larvae of host and pupae was 2.2: 1.0, and 1.3: 3.0, respectively.

Key words: *Phyllocnistis citrella*, *Sympiesis striatipes*, fecundity, longevity, superparasitism, sex ratio, Japan

چکیده

ویژگی‌های زیستی زنبور *Sympiesis striatipes* (Ashmead)، یکی از فراوان‌ترین پارازیتوئیدهای خارجی مینوز برگ مرکبات *Phyllocnistis citrella* Stainton در استان Ehime ژاپن، در شرایط مزرعه و آزمایشگاه مورد بررسی قرار گرفت. متوسط طول دوره‌ی رشدی مراحل نابالغ و طول عمر حشرات بالغ (در ۲۲ الی ۳۱ درجه‌ی سانتی‌گراد، رطوبت نسبی ۷۰-۵۰ درصد و دوره‌ی نوری ۱۲ ساعت روشنایی و ۱۲ ساعت تاریکی) با افزایش دما کاهش یافت و طول عمر حشرات ماده طولانی‌تر از حشرات نر بود. نسبت جنسی نوزادهای پرورش‌یافته در شرایط آزمایشگاهی ٪۸٤/۷ نر و ٪۱۵/۳ ماده محاسبه شد. تخم‌ریزی در حشرات ماده دو تا سه روز بعد از ظهور شروع و تا ۳۹ روز ادامه داشت. متوسط تعداد تخم گذاشته‌شده توسط یک حشره‌ی ماده در طول ۱/۵ ± ۳۳/۸ روز، ۱۲/۹ ± ۱۲۳/٤ عدد (دمای ۱ ± ۲۷ درجه‌ی سانتی‌گراد، رطوبت نسبی ۷۰-۵۰ درصد و دوره‌ی نوری ۱۲ ساعت روشنایی و ۱۲ ساعت تاریکی) با نرخ ذاتی افزایش جمعیت ۰/۳۱۲ محاسبه شد. متوسط مرگ و میر لارو میزبان به روش تغذیه از میزبان و نیش زدن به ازای هر زنبور ماده ٤/۲ ± ٤٤/۷ عدد تعیین گردید. در شرایط مزرعه، ٪۷۵/۸ تخم زنبور ماده روی سن سوم لاروی میزبان و بقیه روی مرحله‌ی پیش‌شفیرگی بود. سوپرپارازیتیسم در شرایط مزرعه ٪۹/۲ تعیین (n = ۲۰۰) و حداکثر هفت تخم روی یک میزبان شمارش شد. در رقابت کشنده‌ی سوپرپارازیتیسم (n = ٤۰)، از ٪۸۷/۵ نمونه فقط یک حشره‌ی کامل زنبور و از بقیه‌ی نمونه‌ها دو حشره‌ی کامل زنبور پارازیت خارج گردید. سوپرپارازیتیسم روی پیش‌شفیره به‌ندرت اتفاق افتاد. نسبت جنسی (M: F) زنبورهای پرورش‌یافته از سن سوم لاروی و شفیرگی در شرایط مزرعه به ترتیب ۱/۰ :۲/۲ و ۳/۰ :۱/۳ تعیین شد.

واژگان کلیدی: *Phyllocnistis citrella*، *Sympiesis striatipes*، باروری، طول عمر، سوپرپارازیتیسم، نسبت جنسی، ژاپن

Introduction

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton, is an important pest of citrus and related Rutaceae in most of the citrus growing regions of the world (Clausen, 1931; Badawy, 1967). Effective chemical control of CLM is difficult because its larva mines between upper and lower surface of the tender leaves, and its pupa is protected by rolled leaf margins (Heppner, 1993).

There is a diverse complex of hymenopterous parasitoids attacking *P. citrella* (Pena *et al*., 1996; Schauff *et al*., 1998), mostly eulophids, and 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 *Sympiesis striatipes* (Ashmead) that is the dominant species in 14 areas in mainland of Japan (Ujiye & Adachi, 1995; Ujiye *et al*., 1996). High levels of parasitism (70%) in the Matsuyama area were caused by seven species of Eulophidae, of which *S. striatipes* was the second abundant species (Mafi & Ohbayashi, 2004). The *S. striatipes* is an ectoparasitoid, polyphagous eulophid that is also found on other gracillariid leafminers such as *Acrocercops* spp. and *Phyllonorycter* sp. in several Asian countries (Kamijo, 1976; Schauff *et al*., 1998).

So far, hymenopterous parasitoids have proven to be successful biological control agents upon several insect pests. However, the biology of majority of these effective parasitoids is not fully understood. Consequently, in this study, we investigated several biological parameters of *S. striatipes* on CLM, including the developmental time at different temperatures, fecundity, sex ratio, adult longevity, host preference, intrinsic rate of natural increase, net reproductive rate, mean generation time and finally superparasitism.

Materials and methods

Different developmental stages of CLM and its parasitoid wasp, *S. striatipes,* were collected from the citrus orchards of Ehime Fruit Tree Experiment Station (33.50° N, 132.42° E, elevation 32 m), Japan. The CLM populations were also maintained in a greenhouse on 2 year-old *Citrus iyo* trees following a methodology described by Smith & Hoy (1995). The adult wasps used in these studies were collected at the pupal stage, reared in Petri-dishes (9 × 2.5 cm) and adult parasitoids were fed with a 70% sugar solution. They were kept at 25°C, 50-70% RH and 12L: 12D photoperiod in an incubator for the different studies (e. g. longevity of adult parasitoid).

Developmental period at different temperatures

Developmental period (egg to adult) of *S. striatipes* was investigated at 22, 25, 28, and 31°C, 50-70% RH and 12L: 12D photoperiod. Female wasps of various ages were randomly collected by aspirators from the field. Citrus leaves infested with third instar larvae (n = 5-10 larvae) were collected from field and placed in Petri-dishes (9 × 2.5 cm). To keep the leaves fresh, wet paper attached to the leaf petioles. Then, two or three female wasps were introduced into each Petri-dish and allowed to lay egg for one day (24 h.). Afterwards, females were removed from Petri-dishes and parasitized hosts were kept in an incubator at the mentioned temperatures. The development of the parasitoid was observed daily until the adult emergence.

Longevity of adult parasitoid and sex ratio

Longevity of adult parasitoids was investigated under laboratory conditions at 22, 25, 28 and 31°C, 50-70% RH and 12L: 12D photoperiod. Newly emerged adults were individually placed in plastic Petri-dishes (9 × 2.5 cm) (10 Petri-dishes for each temperature and three to four adult wasps per Petri-dishes) with droplets of 70% sugar solution as food source. Mortality of parasitoid was recorded daily.

Immature stages of the parasitoid on the CLM larvae and pupae were collected from the field monthly from July to October and reared to adults as above (10 samples per Petri-dishes). The Petri-dishes were kept at 25 ± 1°C and 12L: 12D photoperiod in an incubator. The sex ratio of emerged progenies was separately determined by host stage.

Host stage preference and superparasitism

To determine the preference of host stage and superparasitism, all the developmental stages of CLM larvae and pupa (n = 200 per each stage) were randomly collected from the field. First, the host instar larvae were determined by counting the number of moulted head capsules in the mine under the stereo-microscope. Then, samples were examined to determine the presence of *S. striatipes* eggs. Superparasitism was recognized by counting the numbers of egg and larva of *S. striatipes* on the host instar larvae.

Determination of selected life history parameters

Pre-oviposition, oviposition, and post-oviposition periods, fecundity of the wasp and host mortality were determined by daily observations of individual female parasitoids at 27 ±

1°C, 50-70% RH and 12L: 12D photoperiod. Adult females (n = 10) were offered second and third instar larvae of CLM on detached leaves. Leaves were placed in the transparent plastic boxes (3 × 11 × 13cm) with nylon mesh on one side. One newly emerged female (< 24 h. old) and one male parasitoid was introduced into each box. Parasitoids were fed with small drops of 70% sugar solution 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. Exposed larvae were defined as parasitized if one or more eggs were deposited on or near them. Female parasitoids were maintained for their entire life while the males were replaced as they died. The evidence of stinging and feeding on citrus leafminer larvae ranged from barely detectable markings or very small black dot left after ovipositor insertion. The intrinsic rate of natural increase (r_m), net reproductive rate (R_0) and mean generation time (T) were computed according to the equations given by (Birch, 1948; Carey, 1993).

Data analyses

To determine the effect of various temperatures on the developmental time and longevity of *S. striatipes*, a one-way ANOVA and Tukey's test (P < 0.01) was performed. Other data were analyzed with chi-squared test. JMP (SAS Institute, 2001) was used for statistical analyses.

Results

Developmental period at different temperatures

The mean developmental period of *S. striatipes* reared at different temperatures from egg to adult is summarized in table 1. The developmental period decreased for all stages as the temperature increased. Developmental period from 22 to 31°C ranged from 10.5 ± 0.16 to 6.3 ± 0.12 days in males and 11.0 ± 0.53 to 6.5 ± 0.35 days in females, though no significant difference was found between 25 and 28°C (P > 0.01). There was no significant difference of developmental period between sexes at each temperature (P > 0.05); however, the males developed slightly faster than the females in the entire temperatures applied.

Adult longevity and sex ratios

On a diet consisting of sugar solution (70%), the longevity of female and male parasitoids increased as the temperature decreased (table 2). A significant difference was detected for parasitoid longevity between 22 to 25°C and 28 to 31°C (P < 0.01). Females survived longer than males in the entire temperatures applied. The longest longevity for female and male was 64 and 42 days at 22 °C, respectively. Adult wasps fed only with water or without food died within a few days.

Table 1. Development time (Mean ± SE) of *S. striatipes* at various temperatures on *P. citrella*.

Temperature (°C)	Days from egg to pupa*	Days from pupa to adult*	Days from egg to adult*	
			Male	Female
22	5.1 ± 0.25 (21)	5.5 ± 0.17 (21)	10.5 ± 0.16 (15)a**	11.0 ± 0.53 (6)a**
25	4.4 ± 0.12 (18)	3.6 ± 0.12 (18)	7.8 ± 0.18 (12)b	8.3 ± 0.10 (6)b
28	4.1 ± 0.19 (14)	3.4 ± 0.14 (14)	7.4 ± 0.38 (9)b	7.6 ± 0.25 (5)bc
31	3.1 ± 0.06 (22)	3.3 ± 0.12 (22)	6.3 ± 0.12 (16)c	6.5 ± 0.35 (6)c

*The numbers in the parentheses are the actual number of parasitoid wasp developmental stages.
**Means followed by different letters in the same column are significantly different at *P* < 0.01 by one-way ANOVA and Tukey's test.

Throughout the season, 494 adult parasitoids were reared from the susceptible leafminer stages of third instar larvae and pupae. The emerged parasitoids from the third instar larvae were predominantly males (70.5%), while those from pupae were mostly females (76.0%) (table 3). A x^2 test of independence revealed a dependent relationship (x^2 = 24.8, df = 1, P < 0.0001) between host stage and parasitoid sex every month. The relationship was independent for host stages during the different months (x^2 = 4.14, df = 3, P = 0.2466; x^2 = 1.62, df = 3, P = 0.6554, respectively). The sex ratio (M: F) of the emerged parasitoids from the third instar larvae and pupae was 2.2: 1.0 and 1.0: 3.0, respectively.

Table 2. The effect of temperature (Mean ± SE) on longevity of adult *S. striatipes*, provided with 70% sugar solution.

Temperature (°C)	Average longevity (days ± SE)			
	n	Female	n	Male
22	22	34.8 ± 3.31a**	16	28.9 ± 2.23a*
25	25	27.9 ± 1.72a	22	22.6 ± 2.15a
28	28	20.0 ± 0.57b	16	13.1 ± 0.75b
31	15	14.0 ± 1.10b	15	10.3 ± 0.82b

*Means followed by different letters in the same column are significantly different at *P* < 0.01 by one-way ANOVA and Tukey's test. *n* = sample size.

Host stage preference and superparasitism

The *S. striatipes* laid more eggs on the third instar larvae compared with those laid on the prepupae. No oviposition occurred on the first and second instar or pupal stage of CLM under both field and laboratory conditions. Thus, females preferably laid more eggs on the third instar larvae than the prepupae (x^2 = 227.23, df = 4, P < 0.0001).

Table 3. The percent of *S. striatipes* (male and female) reread to adults from *P. citrella* developmental stages.

Season	From the third instar larva of CLM*	From the pupa of CLM
	Male: Female	Male: Female
July	75% (30): 25% (10)	17.5% (7): 82.5% (33)
August	64.0% (48): 35.0% (27)	23.3% (19): 76.7% (56)
September	64.4% (58): 35.6% (32)	26.7% (24): 73.3% (66)
October	78.6% (33): 21.4% (9)	28.6% (12): 71.4% (30)
Average	70.5 ± 7.4%: 29.5 ± 7.4%	24.0 ± 4.9%: 76.0 ± 4.9
Sex ratio	2.2 (169): 1.0 (78)	1.0 (62): 3.0 (185)

*Number in parentheses are means individual numbers of *S. striatipes*. Average showed by Mean ± SD.

Superparasitism on the third instar larvae was 9.2% (n = 200) under field conditions. The females oviposited up to seven eggs per host larvae under field conditions. In a separate survey, superparasitized host larvae (n = 40) randomly collected from field; 75% of them provided with two eggs, 17.5% with three eggs, and the remainder with four, five, and seven eggs per host larvae. From these samples, single adult parasitoid emerged from 87.5% of the host, and two from 12.5%. Superparasitism was rare on the prepupae.

Life history parameters

Age-specific fertility, host feeding or stinging and survival curve of the female parasitoid on the CLM larvae are shown in figure 1. The *S. striatipes* appeared to be a synovigenic species that matured eggs throughout its adulthood. The presumably mated females began ovipositing 2-3 days after the emergence and the oviposition continued up to the 39 days. Daily reproduction was dependent on the female age and gradually increased until the day eight. Oviposition showed several fluctuations and reduced to low level (0.71 eggs / female per day) by the day 28, and then increased again at the end of oviposition period. The peak of age-specific fecundity was 7.1 ± 1.1 eggs / female per day that observed on the nine day-old females. Individual fecundity varied from 83.0 to 206.0 with mean number of 132.4 ± 13.97 eggs / female. Fertility was quite high (90.0%) with offspring sex ratio accounted for 84.7% males and 15.3% females. The intrinsic rate of natural increase (r_m) was 0.312. The net reproductive rate (R_0) and mean generation time (T) were 69.19 and 13.56 days, respectively. Post-oviposition period was extremely long (6.1 ± 1.58 days) when compared with the pre-oviposition period (0.4 ± 0.16 day). All parasitoids survived for 39 days. Thereafter, the survival rates declined successively and reached zero at the age 41 days. The females survived almost twice as long as the males. Host larval mortality by oviposition was 123.1 ± 12.96 (79 to 190) per female.

Destructive host feeding by adult females began simultaneously with oviposition and ended shortly after parasitoids ceased ovipositing. The *S. striatipes* females exhibited their highest destructive host feeding approximately 17 days after emergence, killing 2.1 host larvae / female per day (fig. 1). Host killed by parasitoid feeding or stinging without oviposition reached 44.7 ± 4.21 per female. Approximately, 26.35% of host larval mortality was caused by host feeding or stinging activity of adult females.

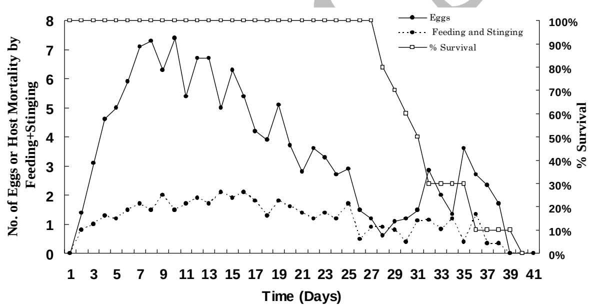

Figure 1. Mean number of daily oviposition, survival and mortality caused by *S. striatipes* (*n* = 10) on its host larvae, *P. citrella* at 27°C.

Discussion

Biological parameters obtained in laboratory and field studies can be used as part of a selection process to identify the best biological control agents for an IPM program (Bellows & van Driesche, 1999). The results presented in this study enlightened the various aspects of the biology of *S. striatipes* under laboratory and field conditions that would be useful for biological control method.

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 study indicated that *S. striatipes* was a typically synovigenic parasitoid, as females produce egg throughout their lifetime (fig. 1). In synovigenic parasitoids, adult nutrition is known to influence the longevity and egg production during the lifetime (Leius, 1961 & 1967). Many synovigenic parasitoids use their host insects either as oviposition sites or as food sources, a behavior called "host-feeding" (DeBach, 1943; Jervis & Kidd, 1986). By feeding upon host, females can improve their longevity, fecundity, and searching efficiency (Syme, 1977).

The developmental time of egg to larva and larva to pupa of *S. striatipes* decreased as temperature increased, and the total developmental period of the male was shorter than the female. The female longevity was significantly longer, compared to that of the male. Similar phenomena observed for *Pnigalio minio* (Walker) and *Cirrospilus vittatus* (Walker), an ectoparasitoid of *P. citrella* (Duncan & Pena, 2000; Urbaneja *et al*., 2002).

The *Pnigalio flavipes* (Ashmead), an ectoparasitoid of *Phyllonorycter elmaella* (Doganlar & Mutuura), preferred tissue feeders (fourth and fifth instar larvae) for oviposition and sap feeders (first-third instar larvae) for host feeding (Barrett & Brunner, 1990). Duncan & Pena (2000) reported that *P. minio*, a polyphagous ectoparasitoids of *P. citrella*, laid eggs on third instar larva with an average of 2.7 times more than those on prepupa. The results obtained here agree with the above mentioned studies. A possible explanation for these phenomena is that the first and second instar larvae are not suitable hosts for development of the parasitoid larva due to lack of enough food source. Avoidance of the leafminer pupae by *S. striatipes* may be due to the hardness of pupal chamber compare with prepupa cell, making ovipositor penetration more difficult. And a shorter developmental period for prepupa compare with third instar larva may allow it to escape from oviposition. Thus, the females preferentially oviposit on the third instar larva, which is recognized as a suitable host for parasitoid larval development.

Many hymenopterous parasitoids are able to discriminate between unparasitized and parasitized host and usually reject the latter for oviposition. This ability was defined as host discrimination (van Lenteren, 1981). Hofsvang (1990) explained the importance of host discrimination such as the prevention of wastage of eggs and hosts (hosts stung frequently have a higher mortality), saving of time, and initiation of migration from a patch with a relative high number of parasitized hosts. Host discrimination by parasitoids is often due to marking pheromones (Rabb & Bradley, 1970) and internal markers (Hofsvang, 1990). This study demonstrated the occurrence of superparasitism in *S. striatipes* through both field survey of a natural populations and manipulative experiment on a laboratory populations. Female *S. striatipes* did not avoid attacking and depositing eggs in hosts parasitized by itself under the laboratory conditions, even though unparasitized hosts were available. Although the behavior of superparasitism in some solitary and gregarious parasitoids is maladoptive in terms of individual offspring fitness, superparasitism can be of advantage in some cases, especially when unparasitized hosts are rare in a habitat patch and mature eggs in the ovaries are abundant (Weisser & Houston, 1993). At present, our knowledge is quite poor over the superparasitism behavior of *S. striatipes* on *P. citrella*. Therefore, additional research is required to clear the obscure aspects of *S. striatipes* superparasitism.

Sex allocation behavior is common among solitary ectoparasitoids and offspring sex ratios are negatively correlated with host size (Askew & Shaw, 1979; Barrett & Brunner, 1990; Ueno & Tanaka, 1997). This correlation is explained by a theoretical model called "host size" (Sandlan, 1979; Charnov *et al*., 1981). The results of this study demonstrated that females *S. striatipes* tended to allocate more female offspring to prepupae of *P. citrella* and males to larval stage. Additional research is required to determine how the host size is estimated by the female wasps across the same larval instar or prepupa for oviposition of different sex eggs.

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