

## Rate of Parasitism of the Antlion Larvae, *Myrmeleon bore* (Neuroptera: Myrmeleontidae) by the Bee Fly, *Villa myrmeleonostena* (Diptera: Bombyliidae)

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**Abstract.** The bee fly, *Villa myrmeleonostena* parasitize the antlion larva, *Myrmeleon bore* living in seaside dune. To obtain the ecological information on the parasitism by *V. myrmeleonostena*, we examined rates of parasitism in the antlion larvae collected monthly from April to October in 1991. Adult bee flies emerged from cocoons, which we obtained by rearing the antlion larvae. The emergence times of parasitoids and hosts were similar. The parasitism rate based on pooled data was the highest in the third instar larvae (9.7%) and the lowest in the first instar larvae (2.5%). More than 15% of third instar larvae collected in April and May were parasitized by *V. myrmeleonostena*. Newly hatched first instar larvae of *M. bore* were found after August, and some of them were also parasitized. From these results, it was inferred that *V. myrmeleonostena* oviposit during the period from late summer to mid-autumn. They probably do not touch an antlion larva to lay an egg on it but might drop an egg while hovering around the pit of antlion.

**Key words:** Parasitoid, parasitism rate, bee fly, antlion larva, *Villa myrmeleonostena*, *Myrmeleon bore*.

### Introduction

Foragers use various biotic and/or abiotic cues to help them locate resources (Bell, 1991). Insectivorous birds generally capture prey very efficiently by using their excellent visual power and they must have accelerated the evolution of cryptic tactics found among numerous species of insects (Edmunds, 1974).

Larvae of antlions such as *Myrmeleon* and *Hagenomyia* make a conical pitfall trap on the surface of sand or fine soil. Particularly the larvae of *Myrmeleon bore*, which live in the seaside dunes, build a clear conical pit on the open field and a pit diameter by the final instar larva can exceed 8 cm (Matsura, 1989). While these pits are easily located, antlions are not subject to predation by predators such as insectivorous birds. This is probably due to the rapid escape into the sand by antlion larvae when they are disturbed (Lucas, pers. com.), and/or their taste might be unpalatable or poisonous for the birds.

Although predators attacking antlion larvae in general have not been found yet, it is known that there exist parasitoids parasitizing them (Bombyliidae, Baba *et al.* (1987); Chalcididae, Steffan (1961) &

Wheeler (1930)). Baba *et al.* (1987) reported that 17 out of 88 cocoons of *M. bore* collected for two years had been parasitized by the bee fly larva, *V. myrmeleonostena*. We have been carrying out the ecological studies on the larvae of *M. bore* living in seaside dunes since 1981 and often reared bee flies from their cocoons. Although prey of the antlion larvae was mainly arthropods dwelling on the surface of dune, they captured sometimes flying-type insects such as hemipterous bugs and ladybird beetles (Matsura, 1986). We have collected numerous prey corpses captured by *M. bore* larvae but found no corpses of *V. myrmeleonostena* among them, which must have come close to the antlions pit to oviposit. The oviposition behavior of *V. myrmeleonostena* has not been observed. Indeed, it is not clear how they oviposit on the antlion larvae successfully without being captured by the host. As a first step in understanding this relationship, we first aimed to obtain the ecological information on the parasitism by *V. myrmeleonostena*, i.e. (i) the age of host parasitized, (ii) rate of parasitism and (iii) season of adult emergence and oviposition in *V. myrmeleonostena*.

## Materials and Methods

The research area was located at Tango Seaside Dunes (134.56°N35.39°E), northern Kyoto Prefecture. We selected three sites, two sandy cultivated fields and a dune slope facing to the Japan Sea, for collecting larvae of *M. bore*. One of these sandy fields (ca. 30 m × 30 m; abbreviated as "CF") was closer from the beach (ca. 200 m) and the other (ca. 50 m × 80 m; "DF") was more distant (ca. 300 m from the beach). These fields were surrounded by forests mainly consisting of pines *Pinus Thunbergii* and locusts *Robinia pseudoacacia* on three sides, and the high density of pits by *M. bore* larvae was found on the sands along boundaries of the forest. We collected the antlion larvae at random once a month from April to October in 1991.

Each antlion larva was kept separately in a cylindrical container (25 mm in diameter, 40 mm high) filled with sand to a depth of ca. 15 mm. The second and third instar larvae were given one chironomid larva (*Chironomus plumosus*) per individual, and one adult of *Drosophila melanogaster* for the first instar larva every three days. Full-grown third instar larvae made a cocoon in the sand, then became a pupa within it. Since the bee fly emerges from a cocoon of parasitized host, we examined cocoons every day to know which species of adults, i.e. *Myrmeleon* or *Villa* would appear. This experiment was conducted under the conditions of  $25 \pm 1^\circ\text{C}$ , 40–60% RH and L : D = 16 : 8.

We determined the parasitism rate of the antlion larvae by the bee fly as a ratio of the number of adult bee flies emerged to the number of cocoons. When neither species of adult emerged from a cocoon, we determined whether it had been parasitized or not by dissecting it. This parasitism rate, however, might be underestimated because an early instar bee fly larva is an endoparasitoid and we cannot distinguish an unparasitized antlion larva from the larva in which the bee fly larva died. To compare the parasitism rate of antlions between third instar larvae and cocoons, we dissected the third instar larvae collected in June and September in 1991 and examined whether they were parasitized by the bee fly larva. When we found an early instar bee fly larva within a host, we recorded where it was located within a host and measured its body length with a micrometer inserted in a binocular microscope.

Throughout the paper, means are given  $\pm$  one standard error.

## Results

### *Development of the bee fly larva and the age of host*

Out of 112 antlion larvae that were dissected 12 larvae contained one early instar bee fly larva per host (Fig. 1). This early instar larva is possibly first instar larva because no larvae were found smaller than this. Mean body length of the bee fly larvae was  $2.42 \pm 0.14$  mm ( $n=7$ ) and they had two long cerci ( $0.33 \pm 0.06$  mm,  $n=7$ ) on the end of abdomen. They seemed to anchor their body with these cerci within a host. Their location within a host body and the number of larvae observed were: dorsal thorax, 1; ventral thorax, 6; lateral thorax, 1; dorsal abdomen, 2; lateral abdomen, 2.

After the antlion larva had pupated within a cocoon, the early instar bee fly larva possibly emerged from the host and became an ectoparasitoid. We deduce this from the fact that both the exuvium of the antlion larva and a corpse of its pupa were found within a cocoon which had been parasitized by the bee fly larva. The final instar larva of *V. myrmeleonostena* was winding itself on the thorax of the pupa of *M. bore* and was sucking body fluid of the host (Fig. 2).



Fig. 1. The early (probably first) instar larva of *V. myrmeleonostena* parasitizing within a host body (*M. bore*).



Fig. 2. The final instar larva of *V. myrmeleonostena* (shown by an arrow) parasitizing a pupa of *M. bore*. It winds itself on the thorax of pupa and sucks body fluid, thrusting its head under a wing pad of the host.

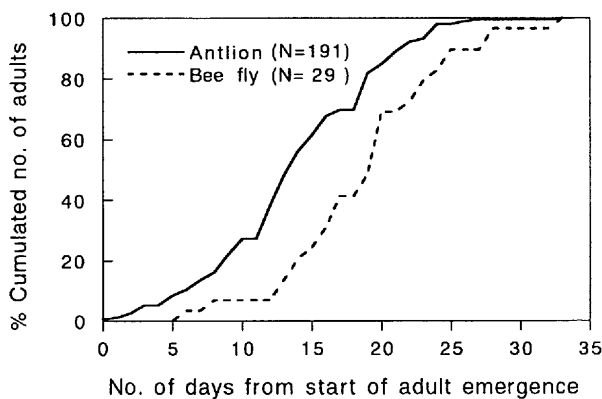


Fig. 3. Periodical changes in the number of adult antlions and bee flies emerged.

Most antlion larvae of *M. bore* became third instar by May in their habitats and formed a cocoon during the rainy season from mid June to mid July. Figure 3 shows the periodical changes in the number of adults of *M. bore* emerged and those of the bee flies emerged from a pupa of *M. bore*, which had been collected at the third instar in May. It shows that the adult emergence of both species was observed nearly at the same season, although the adult emergence of parasitoid was delayed slightly than that of the host. The average number of days from the start of eclosion in the antlions was significantly different between the bee flies and the antlions ( $17.92 \pm 0.92$  days for the bee flies;  $13.94 \pm 0.42$  days for the antlion;  $t = 3.3749$ ,  $P < 0.001$ ).

#### Parasitism rate by the bee fly

We reared 2,686 antlion larvae in total from April to October in 1991 and obtained 2,182 cocoons (cocoon formation rate = 81.2%). Six-point-four percent of cocoons (139/2,182) had been parasitized by

*V. myrmeleonostena*. A small number of cocoons ( $n = 17$ ; 0.78%) had been parasitized by *Lasiochalcidia nigra* (Yasumatsu) (Hymenoptera: Chalcididae). This wasp was a solitary parasitoid as well as the bee fly.

The number of cocoons developed from each instar of antlion larvae and their parasitism rates by the bee fly were shown in Table 1. The first instar larvae of antlions from May to July and the second instar larvae in July were not caught due to their scarceness. The bee flies parasitized all instars of host, and its parasitism rate in pooled data was highest in the third instar larvae (9.72%) and lowest in the first instar larvae (2.47%). The parasitism rate of the third instar larvae was always highest among these instars and it reached more than 15% in April and May. Females of *M. bore* appear to begin to oviposit from the end of July (Matsura, personal observation). Their newly hatched first instar larvae, which occur from August to October, were also parasitized by the bee fly, at a rate less than 5%.

The parasitism rate by the bee fly was different significantly among the collecting sites when all the instar larvae were pooled (Table 2). Although the

Table 1. The numbers of cocoons of *M. bore* obtained in the laboratory and their rates (%) of parasitism by the bee fly (numerals in parentheses).

Month	Instar of antlion larvae when collected			Total
	1st instar	2nd instar	3rd instar	
April	71 (0.0%)	186 (4.3%)	118 (19.5%)	375 (8.3%)
May	0	117 (1.7)	227 (16.3)	344 (11.3)
June	0	32 (0.0)	224 (4.5)	256 (3.9)
July	0	0	116 (5.2)	116 (5.2)
August	220 (3.6)	83 (4.8)	52 (5.8)	355 (4.2)
September	110 (1.8)	202 (8.9)	120 (9.2)	432 (7.2)
October	45 (2.2)	128 (0.0)	131 (4.6)	304 (2.3)
Total	446 (2.5)	748 (4.3)	988 (9.7)	2182 (6.4)

Table 2. Comparison of the parasitism rate of *M. bore* larvae by the bee fly among three collecting sites. All the instar larvae were pooled. See text for the explanation of each site.

	Site		
	DF	CF	Seaside dune
No. of cocoons obtained	1004	577	601
No. of bee flies emerged	62	20	57
% Parasitism	6.2 <sup>a</sup>	3.5 <sup>b</sup>	9.5 <sup>c</sup>

a vs. b:  $\chi^2 = 5.469$  ( $P = 0.0194$ )

b vs. c:  $\chi^2 = 17.451$  ( $P < 0.0001$ )

c vs. a:  $\chi^2 = 5.966$  ( $P = 0.0143$ )

Table 3. Comparison of the parasitism rate of antlions based on dissection of the larvae with that based on the adult emergence of bee flies. Numerals in parentheses show the sample size.

Month of collecting	Stage for judgement		Statistical test
	3rd instar larva	Cocoon	
June	7.14% (70)	4.97% (161)	$\chi^2=2.647$ (N.S.; $P=0.1037$ )
September	16.67% (42)	12.68% (142)	$\chi^2=0.440$ (N.S.; $P=0.5073$ )

parasitism rate of the antlion larvae collected at sea-side dunes was highest, it was not always correlated with a distance from the sea because the antlion larvae at DF (distant field) showed higher parasitism rate than those at CF (close field).

#### Mortality rate of the early instar larva of *V. myrmeleonostena*

To evaluate the mortality rate of early instar larva of *V. myrmeleonostena* living within a body of the antlion larva, we compared the parasitism rate of hosts between third instar larvae and cocoons (Table 3). The rate was slight lower in the cocoons than in the third instar larvae both in June and September. This difference possibly suggests that some early instar larvae had died within the antlion larva before pupation of the host. Since there was not, however, significant difference between them, the mortality rate of bee fly larva as an endoparasitoid is considered to be much low.

## Discussion

The larvae of *V. myrmeleonostena* changed its life-style from endoparasitoid to ectoparasitoid with its development. Parasitoids that show this type of change in their life histories are minority among insect parasitoids (Godfray, 1994). This change in the feeding behavior would be caused by the character of host, antlion larva. Since the larvae of *M. bore* live in dry sand, an ectoparasitoid must be easily scoured with sand grains even though it attaches itself to the host. After the host pupated within a cocoon, the larva of *V. myrmeleonostena* must have emerged from the host and sucked body fluid of the unresistant and soft-bodied pupa from its outside. It is unclear why the older larva changes into ectoparasitoid, but external feeding on the host might be more efficient than internal feeding for a grown larva which needs more amount of oxygen for respiration.

Cocoon formation of the host, *M. bore* is mainly carried out in a rainy season (mid June to mid July), and the adults emerge less than one month after cocoon formation (Matsura *et al.*, 1991). In addition,

present study revealed that adults of *V. myrmeleonostena* had emerged nearly at the same period as *M. bore* (Fig. 3). Therefore, the adult eclosion of *V. myrmeleonostena* also would be found in mid summer in the field, while they can potentially emerge in every season under the conditions of a long daylength as confirmed in this research. First instar larvae of the antlions are found at high density in September and October in their habitats (Matsura *et al.*, 1991). Since the parasitism of the first instar larvae was observed only in the larvae collected in August to October, we can infer that the bee flies oviposit during this period.

The rate of parasitism of antlion larvae was higher in order of third instar > second instar > first instar, and was highest particularly in the third instar larvae collected in April or May. If a survival rate is higher in the antlion larvae parasitized by the bee fly than unparasitized larvae, then the rate of parasitism would become higher with a development of hosts. It is known that parasitoids often manipulate their host insects through physiological or behavioral mechanism in order to raise their fitness (Vinson & Iwantsch, 1980; Godfray, 1994). For example, braconid wasp *Cotesia congregatus* attacking the tobacco hornworm *Manduca sexta*, can cause the development of supernumerary larval instars and prevent metamorphosis by suppressing the normal drop in host juvenile hormone to make the host to remain longer in the larval feeding stage (Beckage & Riddiford, 1982). There are some examples showing that parasitized hosts reduced the risk of predation due to its sluggishness, compared to unparasitized hosts (Fritz, 1982). Palmer (1982) investigated the behavior of bee flies *Anthrax gideon* parasitizing the larvae of tiger beetles and discovered that the survival rate of third instar larvae of the host was significantly higher than that of unparasitized host. He could compare them directly, because *A. gideon* is an ectoparasitoid throughout its larval period unlike *V. myrmeleonostena*. In the case of *M. bore* larvae, we could detect neither morphological or behavioral difference between parasitized and unparasitized larvae. To confirm host manipulation by an endoparasitoid such as *V. myrmeleonostena*, we would need to prepare another experimental

design.

How *V. myrmeleonostena* parasitize the antlion larva waiting for prey at the base of pitfall trap? Females of several subfamilies of Bombyliidae possess a 'sand chamber' on their abdomen, in which eggs are individually coated with sand, and the females oviposit by flicking coated eggs with a vigorous motion of the abdomen while hovering (Yeates, 1994). This characteristic organ would enable bee flies to parasitize various predacious insects. Larvae of tiger beetles are vigorous predators sitting in a burrow dugged on the ground like an antlion larva. According to Palmer (1982), the females of bee fly *Anthrax gideon* and *A. analis* hovers 3 to 5 cm from the burrow of larva of tiger beetle and darts rapidly about 1 cm toward the burrow. It releases a single egg coated by soil particles at the point nearest the hole. That is, they do not lay eggs on the host body directly but their hatched larvae reach the host by themselves. *V. myrmeleonostena* may also adopt a similar oviposition behavior as *Anthrax* bee flies. Therefore, to observe the oviposition behavior of *V. myrmeleonostena* in the field, we should await them coming to the pits of antlion larvae in late summer to mid-autumn, because they possibly spend little time (almost instantaneous) on oviposition. The reason why we have never found the bee flies among prey of antlion larvae collected for 2 years (e.g. Matura, 1986) would be caused by their characteristic oviposition behavior.

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