



## Biology, rearing and field release on Guam of *Euplectrus maternus*, a parasitoid of the fruit-piercing moth, *Eudocima fullonia*

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**Abstract.** The eulophid parasitoid, *Euplectrus maternus*, is a gregarious ectoparasitoid of the larvae of the fruit-piercing moth, *Eudocima (Othreis) fullonia*. This parasitoid is indigenous to India and was released into Guam in 1998–99 to aid in the biological control of *E. fullonia*, an important pest of ripe guava, banana, mango, pomegranate, litchi, papaya, tomato, orange and other fruit crops. In the laboratory, biology and rearing of *E. maternus* on the larvae of *E. fullonia* were studied. In no-choice tests, oviposition by *E. maternus* was significantly greater on first and second instar larvae than on third instar larvae. However, in free-choice tests, oviposition by *E. maternus* was significantly greater on second instar larvae, followed by first and third instar larvae. This parasitoid did not lay eggs on later instars, either in no-choice or free-choice tests. Females laid a significantly greater number of eggs (>80%) on the dorsal surface of the larvae than on either lateral or ventral surfaces (1 to 8%). In general, more eggs were laid on abdominal segments 1–3 on larval instars one to three. The survival of the immature stages (from egg to adult) of *E. maternus* on second instar *E. fullonia* larvae was 61%, which was significantly greater than the survival rates on first and third instar larvae (32% and 26%), indicating that second instar host larvae are ideal for mass rearing of the parasitoid. Mated female *E. maternus* continuously laid eggs on the second instar larvae of *E. fullonia* for up to 30 days, but the greatest number of eggs were laid during the first week after exposure. When *E. maternus* laid more than two eggs on host larva, more female progeny were produced, indicating female-biased reproduction. Adult parasitoids lived longer when fed with a honey: water (50% w/v) solution than with pure honey. Similarly, the fecundity of females increased significantly when fed with the honey-water solution when compared to feeding with pure honey. To date, *E. maternus* has not established in Guam.

**Key words:** biology, Eulophidae, *Eudocima (Othreis) fullonia*, *Euplectrus maternus*, Hymenoptera, Lepidoptera, Noctuidae

### Introduction

The fruit-piercing moth, *Eudocima (Othreis) fullonia* (Clerck) (Lepidoptera: Noctuidae), is an important pest of orange, banana, sour sop, litchi, tomato,

grapes, pomegranate, mango, eggplant and other fruits in the subtropical and tropical regions of Africa, Asia, Australia and the Pacific (Waterhouse and Norris, 1987). *E. fullonia* is considered to be one of the top ten pests in the Pacific region. Adult moths pierce and suck the sap of ripe fruits at night. Vines belonging to the family Menispermaceae in Africa, Asia and Australia and *Erythrina* spp. (Fabaceae), as well as menisperms, in the Pacific are larval hosts (Muniappan et al., 1995).

Parasitoids observed to attack *E. fullonia* include the egg parasitoids *Telenomus* sp., *Ooencyrtus* sp. and *Trichogramma* sp. in Micronesia (Muniappan et al., 1993) and the rest of the Pacific (Sands et al., 1993); the larval parasitoids, *Winthemia caledoniae* Mesnil, *Euplectrus platyhypenae* Asmead, *Echthromorpha striata* Krieglner and *Lissopimpla pacifica* Marley, in New Caledonia (Cochereau, 1977), *Winthemia* sp. in American Samoa (Hoyt, 1955), *Carcelia iridipennis* (Wulp) and *Euplectrus* sp. in Indonesia (Bezzi, 1925; Van der Laan, 1981), *E. platyhypenae* in Hawaii (Heu, 1985) and Fiji (Maddison, 1982), *Euplectrus melanocephalus* Girault in Australia (Jones and Sands, 1999), *Euplectrus maternus* Bhatnagar (Hymenoptera: Eulophidae) in India (Bhatnagar, 1951); and two pupal parasitoids, *Trichospilus diatraeae* Cherian and Margabandhu and *Brachymeria* sp., from Guam (Muniappan et al., 1993).

*Winthemia caledoniae* from New Caledonia was introduced to Tonga in 1979 and to Fiji in 1983-84 for biological control but did not establish (Waterhouse and Norris, 1987). The egg parasitoids *Telenomus* sp. Nixon and *Ooencyrtus* sp. from Papua New Guinea were introduced to Samoa in 1988 and Fiji in 1990. Both parasitoids established in Fiji but only *Telenomus* sp. established in Samoa (Sands et al., 1993).

Denton et al. (1999) found *E. fullonia* to be a serious problem in Guam in spite of a high percentage of egg parasitism. Sands (1996) recommended that *E. maternus*, a larval parasitoid of *E. fullonia* and *E. materna* in India, be considered for introduction into Pacific countries where *Eudocima* species are pests and no effective larval parasitoids exist.

Four species of *Euplectrus* have been utilized for classical biological control of various noctuid pests. *Euplectrus platyhypenae* was introduced to the Hawaiian Islands from Mexico against *Spodoptera mauritia* (Boisd.) and other armyworms (Swezey, 1924; Osborn, 1938). From Hawaii, *E. platyhypenae* was introduced to the Philippines against *Spodoptera litura* (Boisd.), but did not establish (Uichanco, 1934). It was also introduced to Fiji, from Hawaii, against *Levuana iridescens* Bethune-Baker in 1925 (Rao et al., 1971). *Euplectrus laphygmae* Ferriere was introduced from East Africa to Israel during 1969-1970 to control *Spodoptera littoralis* (Boisd.). *Euplectrus putleri* Gordh was introduced from Colombia, South America in

1975 against *Anticarsia gemmatalis* (Hübner) in the U.S.A., and established (Puttler et al., 1980; Waddill and Puttler, 1980). Likewise a *Euplectrus* sp. was introduced from India to Guam in 1986–87 and established against *Penicillaria jocosatrix* Guenee (Nafus, 1991). Parasitoids in the genus *Euplectrus* are multivoltine, gregarious ectoparasitoids of the larvae of Lepidoptera, (Gerling and Limon, 1976), mainly Noctuidae (Prinsloo, 1980). Females sting host larvae before ovipositing causing temporary paralysis. The venom injected while stinging arrests larval-larval ecdysis, a common phenomenon in this genus (Coudron, 1991). The biology of *E. laphygmae* in South Africa (Neser, 1973) and Israel (Gerling and Limon, 1976), *E. puttleri* in the United States (Puttler et al., 1980) and *E. melanocephalus* in Australia (Jones and Sands, 1999) has been reported.

One of us (RM) undertook an exploratory trip to India in August 1997 to collect and confirm the identity of *E. maternus*. Parasitoid specimens were collected from Coimbatore, Bangalore and Pune city areas in India. Dr. M.E. Schauff of the U.S. National Museum confirmed the identity. Two trips were made to India in 1998 and 1999 to collect the parasitoid and to start cultures for research in Guam. Herein, we discuss the biology, culture and field release of *E. maternus* in Guam.

## Materials and methods

### *Parasitoid populations*

In India from August through early November, a few months after the onset of the monsoon season, menisperm plants grow vigorously and many *E. fullonia* larvae are found on them. Surveys were done in the southern and western parts of India in the states of Andhra Pradesh, Gujarat, Karnataka, Maharashtra, and Tamil Nadu during October 1998 and October–November 1999. Menisperm vines were found mostly along fence lines. As early instar larvae are known to be parasitized by *E. maternus*, the tender shoots of the menisperms were searched and parasitized larvae collected into test tubes (20 cm × 2.5 cm) for transportation to the laboratory. The common species of menisperms found in the plains of India were *Cocculus hirsutus* (L.) and *Tinospora cardifolia* (Willd.) supporting *E. fullonia*, *Eudocima materna* (L.) and *Eudocima homaena* Hübner. *Euplectrus maternus* parasitized all three species of *Eudocima*. Host plants of the species, *Cyclea peltata* (Lam.), *Stephania japonica* (Thunb.) and *Pachygone ovata* (Poir.), were common at high altitudes (1000 to 1500 m) in the Kodugu district of Karnataka state. Even though these species supported *Eudocima* spp., the parasitoid *E. maternus* was absent at the higher altitudes. Larvae collected in the field

were checked for parasitoids with a hand lens and examined further in the laboratory under a dissection microscope.

#### *Importation*

A shipment of *E. maternus* was hand-carried to the Western Pacific Biocontrol Quarantine Laboratory (WPBQL) at the University of Guam in November 1999, and a successful parasitoid culture was established. This shipment consisted of parasitoids raised from *E. fullonia*, *E. materna* and *E. homaena* collected from *Cocculus* sp. and *Tinospora* sp. from Coimbatore, Bangalore, and Raichur in India. Parasitoids were reared in a laboratory in Bangalore to purify cultures of hyperparasitoids that may have been present. Subsequent rearing at the WPBQL confirmed the absence of hyperparasitoids.

#### *Rearing of host and parasitoid*

Adults of *E. fullonia* were placed in field cages (1.8 m × 1.8 m × 1.8 m) outdoors. A 1.5 m tall *Erythrina variegata* L. potted plant was kept inside the cage for oviposition by the moths. The potted plant in the cage was replaced once every two weeks. Moths were fed orange, papaya, banana and other fruits that were hung from the roofs of the cages. *E. fullonia* eggs were collected from the caged plant leaves every morning and kept in test tubes (20 cm × 2.5 cm) with the open end sealed with a muslin-covered cotton plug. Newly emerged larvae were transferred from the test tubes to plastic boxes (8.0 cm in height × 11.7 cm in diameter), which had previously been thoroughly washed with soap and water, rinsed with 10% bleach, and dried. For aeration and to prevent excessive humidity inside the container, a 2-cm diameter hole was made in the center of each container and covered with muslin cloth. Larvae were fed tender leaves of *E. variegata*. To provide a continuous supply of various host stages, a number of such plastic boxes were prepared and placed in a laboratory with temperatures varying between 26 and 30 °C and 75 to 85% relative humidity (RH) under a photoperiod of 16 hours light (L) and 8 hours dark (D). Later instar larvae were transferred to a plastic container (27 cm × 34 cm × 10 cm) fitted with a 2-cm wire mesh false floor. This allowed fecal material to drop to the base of the container while eliminating fecal contamination of the leaves supplied for food. In this container, a window of 15 cm × 20 cm was cut in the middle of the lid and covered with muslin cloth to allow adequate ventilation. Pupae were transferred to an identical container without the false floor, for the emergence of the adults. This procedure consistently produced large numbers of various stages of the host, *E. fullonia*.

Parasitoids were cultured in the laboratory at a constant temperature of  $28 \pm 2^\circ\text{C}$  and 75 to 85% RH under a photoperiod of 16L:8D. Newly emerged adult male and female parasitoids were kept together in a test tube for 24 h to facilitate mating, even though the females usually mate immediately after emergence. Since later instars (fourth, fifth and sixth) of the host do not appear to be readily parasitized by this parasitoid (Bhumannavar and Virak-tamath 2000, R. Muniappan, person. observ.), individual first, second and third instar larvae of *E. fullonia* were exposed to a mated female parasitoid in a test tube containing a streak of honey:water solution (50% w/v) for the adult parasitoid feeding and a piece (1.5 cm<sup>2</sup>) of *E. variegata* leaf for host larval feeding. The tubes were examined in the morning and evening for oviposition by the parasitoids. Larvae with parasitoid eggs were removed and a new larva of *E. fullonia* was provided. Parasitized larvae were kept in individual test tubes closed with a cotton plug. A small piece of fresh *E. variegata* leaf was provided every day until the larvae died. When the parasitoid larvae pupated, the tube was cleaned of the leaf debris, larval webs and fecal material, and parasitized host larvae placed again in the respective tubes for emergence of the parasitoids. Upon emergence of the adult parasitoids, some were used for further replication and tests while others were field-released. Adults were sexed by closely observing the ventral surface of the tip of the abdomen in a clean test tube under the microscope. Females can be identified by the presence of two small, rounded brown spots at the base of the ovipositor.

#### *Host larval instar preference*

Studies were conducted on the six larval instars of *E. fullonia* using no-choice and free-choice tests in a laboratory with room temperature of  $28 \pm 2^\circ\text{C}$  and 75 to 85% RH under a photoperiod of 16L:8D. In no-choice tests, a parasitoid's oviposition response to different host instars was tested using single *E. fullonia* larvae. Early instar larvae (first, second and third) were kept individually in test tubes (20 cm  $\times$  2.5 cm) while later instar (fourth, fifth and sixth) larvae were placed individually in plastic boxes (8 cm height  $\times$  11.7 cm diameter). One mated female parasitoid was introduced into each test tube or plastic container with a streak of honey:water solution (50% w/v) for adult parasitoid feeding, and a piece of *E. variegata* leaf (1 cm  $\times$  1.5 cm for early instars or whole leaf for later instars) for host larval feeding. The honey:water solution and leaf pieces were changed once every two days or when the leaves were eaten. Parasitized larvae were removed and the numbers of eggs laid on different thoracic and abdominal segments of each instar larvae observed under a dissection microscope and noted. The egg positions on dorsal, ventral and lateral sides of the segments were also recorded. The parasitoid preference to different host instar larvae was also studied under

free-choice using a test tube (20 cm × 2.5 cm) for the first three instars and a plastic container (8 cm height × 11.7 cm diameter) for the three older instar stages. One of each newly molted first to third instar or fourth to sixth instar larvae of each were placed either in a test tube or a plastic container along with one mated female parasitoid. The parasitized larvae were removed on the following day, and the number of eggs on each larva was counted under a microscope. Whenever a larva molted or was parasitized it was replaced with a new larva and similar counts were continued until the parasitoid died. Both no-choice and free-choice tests were repeated six times.

*Survival of immature stages of E. maternus on early instar larvae of E. fullonia*

One newly molted first to third instar of *E. fullonia* was individually placed in a test tube (20 cm × 2.5 cm) with one mated female *E. maternus* parasitoid. Host larvae and adult parasitoids were fed as previously described. Parasitized larvae or the larvae that had molted in the subsequent instar stage were removed, and newly formed larvae of the same instar were provided until the death of the parasitoid. The number of eggs laid on each host instar and the number of adult parasitoids that emerged from the respective larvae were recorded. The experiment was replicated six times for each instar.

*Ovipositional pattern and longevity of E. maternus females on early E. fullonia instars*

We observed that most of the *E. maternus* parasitoids oviposited selectively on the second instar larvae of *E. fullonia*. Therefore newly-formed second instar larvae of *E. fullonia* were placed individually in a test tube (20 cm × 2.5 cm) with one mated female parasitoid. Host larvae and adult parasitoids were fed as previously described. The parasitized larvae were removed the next morning, and counts were made of the number of eggs laid on the larvae, using a microscope. Parasitized larvae or larvae molted into the next instar stage were replaced with new larvae and similar counts were made until the parasitoid died. This experiment was replicated six times.

*Sex ratio of E. maternus on E. fullonia*

Several second instar *E. fullonia* larvae were exposed daily to *E. maternus* during a period of three months. Parasitized larvae were separated and kept in individual test tubes (20 cm × 2.5 cm) until emergence of parasitoids. Emerged parasitoids were sexed and sex ratio was recorded for the number of parasitoid eggs laid on each larva.

*Longevity and oviposition rate by E. maternus feeding on pure honey and honey/water*

Newly-molted second instar larvae of *E. fullonia* were each placed in a test tube (8 cm × 11.7 cm) and one male and female parasitoid was introduced with a streak of honey:water solution (50% w/v) and pure honey (100%) for adult parasitoid feeding and a piece (1 cm × 1.5 cm) of *E. variegata* leaf for larval feeding. Similarly, in another experiment, a test tube with one larva and one mated female parasitoid was provisioned with a streak of honey:water 50% (w/v) pure honey and a piece of *E. variegata* leaf. The respective honey:water solution or pure honey and leaf pieces were changed once every two days while larvae were replaced daily. Observations were made on the longevity of male and female parasitoids and the number of eggs laid was also recorded. This experiment was replicated six times.

*Field release*

Periodic releases of the adult *E. maternus* parasitoids were made in fields at the Mangilao Golf Course, located in the northeastern part of Guam, and near hotels at Ypao Beach on the northwestern side of the island, starting from June 12, 1999 and continuing through September 11, 2001 as these sites had clusters of *Erythrina* trees. Trees were examined and releases were made only on the trees that had early instar larvae of *E. fullonia*. In other parts of the island, *Erythrina* trees are scattered and sparse. A total of 5059 (2208 males and 2851 females) were released during the 27-month period. First, second and third instar larvae of *E. fullonia* were collected on a weekly basis near the parasitoid release sites as well as other sites from May 10, 2000 to June 2002 and examined for eggs of the parasitoid under a magnifying lens in the laboratory to determine the field establishment of the parasitoid. At each location, all available *E. fullonia* larvae were collected and observed for eggs or larvae of the parasitoid. The larvae collected per week ranged from 50 to 115.

*Statistical analysis*

All data were analyzed using SPSS 10.0 for Windows. The data from choice experiments of the host larval preference studies was analyzed using the repeated measure ANOVA while the data from the no-choice oviposition experiments, from the distribution of moth eggs on the parasitoid, oviposition site preference, survival rate and oviposition pattern of the parasitoid, were analyzed using analysis of variance. The data on parasitoid longevity was analyzed using a two-way ANOVA with treatment and sex as factors and means were separated using the LSD test.

Table 1. Mean number of eggs laid by *Euplectrus maternus* on different host larval stages of *Eudocima fullonia*

Host stage	No. of eggs laid (Mean $\pm$ SE)	
	No-choice	Free-choice
First	36.2 $\pm$ 2.3a <sup>(1)</sup>	13.0 $\pm$ 3.2b
Second	38.8 $\pm$ 1.8a	65.6 $\pm$ 2.4a
Third	22.0 $\pm$ 2.4b	3.4 $\pm$ 0.6b
Fourth	0.0c	0.0c
Fifth	0.0c	0.0c
Sixth	0.0c	0.0c

<sup>1</sup>In each column values that are followed by the same letter are not significantly different from each other ( $P < 0.05$ ; LSD test).

## Results

### *Parasitoid host larval instar preference*

When different stages of host larval instars were exposed to female parasitoids under no-choice and free-choice experiments, no eggs were laid on later instars (fourth to sixth) (Table 1). In no-choice test, *E. maternus* parasitoids laid significantly more eggs ( $F = 6.26$ ;  $df = 1,5$ ;  $P < 0.05$ ) on first and second instar larvae than on third instar larvae. In free-choice tests, parasitoid oviposition on second instar larvae was 4 times and 20 times greater than on first and third instars, respectively. Emergence of *E. maternus* from *E. fullonia* parasitized in the second instar larvae was 61%, significantly greater than the emergence from host larvae parasitized in the first and third instars larvae (32% and 26%, respectively) ( $F = 10.14$ ;  $df = 1,5$ ;  $P < 0.05$ ; Figure 1). There was a natural mortality of 39% of eggs laid on the second instar host. However, a natural mortality of 32% of eggs laid and 36% mortality of parasitoid larvae during development was noted in the first instar, and 34% natural mortality and 40% mortality for eggs and parasitized larvae, respectively, was observed on the third instar host. Some times the parasitoid host fed on the oviposited larvae, resulting in early death of the host and the parasitoids. The number of eggs laid by a parasitoid on a second instar larva ranged from one to nine. The sex ratio of parasitoids that emerged from the eggs laid on a larva varied depending on the number of eggs laid. The sex ratio of the parasitoid emerged from the larvae with one to two eggs was 0.50 ( $n = 10$  for one egg and  $n = 21$  for two eggs), larvae with three eggs was 0.33 ( $n = 26$ ), four eggs was 0.30 ( $n = 24$ ), five eggs was 0.22 ( $n = 30$ ) and six eggs was 0.20 ( $n = 18$ ).

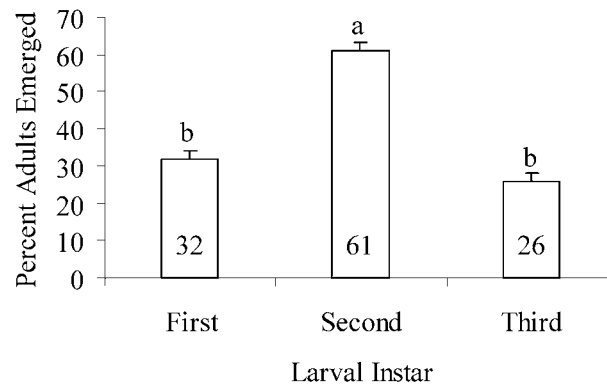


Figure 1. Emergence (Mean  $\pm$  SE) of *Euplectrus maternus* from *Eudocima fullonia* parasitized in the 1st–3rd instars. Bars with identical letters are not significantly different from each other ( $P < 0.05$ ; LSD test); Values within the bars indicate percent *E. maternus* adults emerged from *E. fullonia* larvae.

Table 2. Distribution of eggs deposited by *Euplectrus maternus* on different segments of the thorax and abdomen of *Eudocima fullonia*

Host stage	Percent of eggs laid (Mean $\pm$ SE)												
	Thorax			Abdomen									
	S1	S2	S3	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
First	0	0	8	12b <sup>1</sup>	22b	31a	11	3	0	0	0	0	0
Second	1	3	4	25a	37a	22b	2	1	1	2	2	0	0
Third	0	2	7	13b	18b	16c	3	1	0	1	2	0	0

<sup>1</sup>Values within columns followed by the same letter are not significantly different from each other ( $P < 0.05$ ; LSD test). S: segment.

High mortality of the host larvae and parasitoids was observed when there were ( $\geq 7$ ) eggs laid on a single larva.

#### *Parasitoid oviposition behavior and fecundity*

In the present study, parasitoids were seen to crawl around the preferred instar larvae. When the female parasitoid was ready to lay eggs on a larva, it hovered over the host, and then landed directly on it. The female then inserted the ovipositor into the integument, resulting in temporary paralysis of the larva, and then oviposited. *Euplectrus maternus* eggs were laid from the third thoracic segment to the fifth abdominal segment on first instar *E. fullonia*, with a maximum number eggs laid on the third abdominal segment (Table 2). On second instar larva, eggs were laid on all three thoracic segments and

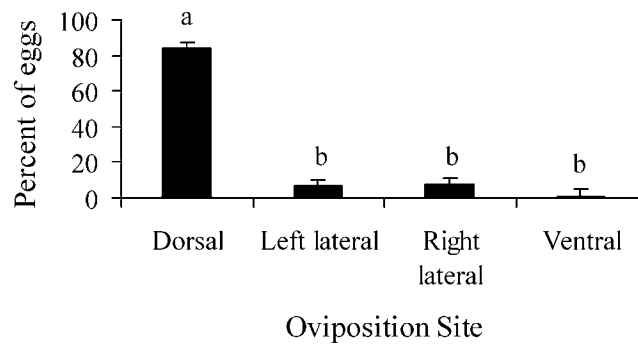


Figure 2. Oviposition site preference (Mean  $\pm$  SE) by *Euplectrus maternus* on early instar larvae of *Eudocima fullonia*. Bars with the same letters are not significantly different from each other ( $P < 0.05$ ; LSD test).

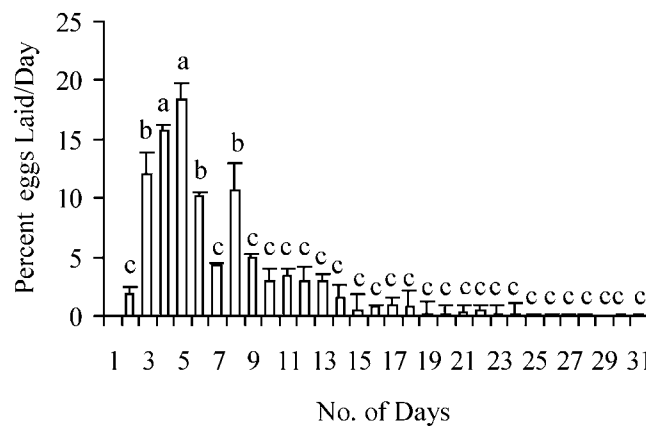


Figure 3. Percent of eggs laid per day by *Euplectrus maternus* on 2nd instar larvae of *Eudocima fullonia*. Mean percentage  $\pm$  SE obtained from 6 replicates, each replicate consisted of one insect. Bars with the same letters are not significantly different from each other ( $P < 0.05$ ; LSD test).

abdominal segments 1–8, with a maximum number of eggs laid on the second abdominal segment. On third instar larva, eggs were laid on the second and third thoracic segments and abdominal segments 1–8, with most eggs laid on the second abdominal segment. In general, eggs were preferentially laid on the abdominal segments 1–3 on all three larval instars (Table 2). The pooled analysis indicates that the parasitoid laid significantly more eggs (>80%) on the dorsal side of the larvae than on either lateral or ventral sides (1 to 8%) ( $F = 12.21$ ,  $df = 1,5$ ;  $P < 0.05$ ; Figure 2). Mated *E. maternus* females continuously laid eggs on second instar *E. fullonia* larvae for up to 30 days, although 62.5% of their total reproduction occurred during the first week after exposure. *E. maternus* females laid significantly more (Figure 3) eggs

on days 4 and 5 after the first exposure, preferring days 3, 6, 7, 8 after this ( $F = 10.56$ ;  $df = 1,5$ ;  $P < 0.05$ ). Adult parasitoids lived longer when fed with honey:water solution than with pure honey alone. Both females and males lived significantly longer (mean of 14.5 and 13.5 days) when fed with the honey:water solution than the ones fed with pure honey (7.0 and 8.2 days). A mean number of 22.2 eggs was laid by mated females fed with the honey:water solution, which is significantly higher than the number of eggs laid when females were fed with pure honey (mean of 15.2 eggs) ( $F = 14.13$ ,  $df = 2,5$ ;  $P < 0.05$ ).

#### *Field establishment*

No parasitoid eggs or larvae have been recovered from the *E. fullonia* larvae which were collected on a weekly basis from release sites.

### **Discussion**

Our results indicate that in the laboratory *E. maternus* lays eggs on first, second and third instar larvae of *E. fullonia* but not on fourth, fifth and sixth instars. These results agree with those of Bhumannavar and Viraktamath (2000) who reported that in the field *E. maternus* parasitized first and second instar larvae but rarely third instar larvae. Oviposition behavior on different instars of larvae is reported to vary within the genus *Euplectrus*. For example, *E. laphygmae* lays eggs on the first four of the six larval stages of *S. littoralis* in Israel (Gerling and Limon, 1976) while the same parasitoid lays eggs on second to fifth instar larvae of *Plusia acuta* Wlk. in South Africa (Neser, 1973). *E. puttleri* lays eggs on all six larval instars except the first instar of *A. gemmatalis* in Missouri (Puttler et al., 1980) while *E. melanocephalis* utilizes the first four instars of the larvae of *E. materna* in Australia (Jones and Sands, 1999). Nafus (1991) found a *Euplectrus* sp. parasitizing *P. jocosatrix* on Guam lays eggs on all five instars, although the first and fourth instars were parasitized less frequently and the fifth instar was seldom parasitized. Most species of *Euplectrus* laid more eggs on older, rather than younger, host instars (Neser, 1973; Puttler et al., 1980; Uematsu, 1981; Nafus, 1991) except *E. melanocephalis* and *E. maternus* which preferred earlier instars.

The number of *E. maternus* adults produced was greatest from the second instar, followed by first and third instar larvae of *E. fullonia*. There was a natural mortality of 39% of the eggs laid on the second instar host. However, an additional 7% mortality was noted for eggs laid on in the first instar. We suggest this is a result of the feeding on the host by the adult parasitoid after oviposition and the inability of the early and late first instar hosts to sustain

development of more than two and three parasitoid larvae, respectively. A mortality of 61% was observed during the development of the parasitoid from egg to pupal stage on the third instar host. This could be due to enlargement of the parasitoid eggs and larvae getting tangled in the webbing of the host larvae and then being dislodged or having the host larvae dislodge the eggs and parasitoid larvae with their mouthparts when those eggs were laid on the distal end of the abdominal segments. Thus, it is evident that the second instar *E. fullonia* is the preferred host for the parasitoid *E. maternus* because the highest survival rate of the parasitoid eggs was observed in this instar, suggesting second instar hosts would be the most suitable stage for mass rearing *E. maternus*.

We showed that *E. maternus* eggs were laid between the third thoracic segment and the eighth abdominal segment of the first to third instars of *E. fullonia* larvae. A maximum number of eggs was laid on the second abdominal segments although in general, eggs were laid on abdominal segments 1–3 of all three larval instars. Bhumannavar and Viraktamath (2000) found eggs on the first three abdominal segments in field-collected *Eudocima* spp. larvae. Jones and Sands (1999) noted that *E. melanocephalus* laid eggs on abdominal segments 1–9, with 90% of the eggs having been laid on the second and third abdominal segments of the larvae of fruit-piercing moths in Australia. Uematsu (1981) noted that *E. kuwanae* laid 93% of its eggs on the first three abdominal segments of *Argyrogramma albostrigata* (Bremer et Grey) while Nesar (1973) observed that *E. laphygmae* laid 95% of its eggs on the first three abdominal segments of *P. acuta*. Wall and Berberet (1974) found that *E. platyhypenae* placed eggs anterior to the fourth abdominal segment. It is probable that the survival of the eggs and larvae of *E. maternus* is highest when placed on the first three abdominal segments of the host as the host larvae cannot reach these parts of the body with their mouthparts to remove them. In the present study the parasitoid laid over 80% of the eggs on the dorsal side of the larvae, and laid very few eggs laterally or ventrally. Bhumannavar and Viraktamath (2000), Jones and Sands (1999) and Nesar (1973) reported similar findings. We observed that eggs positioned on the dorsal side of the first three abdominal segments were dislodged less frequently than those laid on the ventral side, or those laid at various positions on the distal segments of the abdomen.

From the present study it was found that the sex ratio in *E. maternus* was female-biased, as observed frequently in gregarious parasitoids (Godfray, 1994). According to Hamilton (1967) a female-biased sex ratio should result in a strong local mate competition. Because only female parasitoids kill hosts directly by oviposition and host feeding, female-biased sex ratio in may contribute to increase host mortality in *E. fullonia* population.

We observed that both sexes of adult *E. maternus* parasitoids lived longer when the adults were fed with the 50% honey:water solution than when the adults were fed with pure honey, in agreement with Bhumannavar and Viraktamath (2000). Similarly, Puttler et al. (1980) reported that *E. puttleri* females and males fed with 50% honey solution at 30 °C lived five and seven times longer, respectively, than ones fed with pure honey. Also in our experiment, the fecundity of mated females was increased significantly when fed with honey:water compared with pure honey. Bhumannavar and Viraktamath (2000) reported that the number of eggs laid by *E. maternus* when fed with dilute honey ranged from 65–78 per female parasitoid. We observed the females to mate only once as also reported by Gerling and Limon (1976) in case of *E. laphygmae*.

Sands (1996) hypothesized that *E. maternus* might be valuable for introduction into countries where *Eudocima* spp. is a pest. We found that *E. maternus* parasitoids released at two different field locations in Guam did not establish even 10 months after the release, indicating a need for careful consideration of laboratory findings with regard to the potential in-field performance of a natural enemy. It is likely that *E. maternus*, a parasitoid which was introduced from India where it parasitized *Eudocima* spp. that fed on Menipermaceae vines has not adapted to parasitizing *E. fullonia* on Guam where *E. fullonia* feeds on *Erythrina* spp. To date, introductions of *E. platyhyphenae* in Hawaii, *E. laphygmae* in Israel, *E. puttleri* in southern U.S.A and a species of *Euplectrus* in Guam have been promising. Waddill and Putler (1980) found satisfactory field establishment of *E. puttleri* on the velvetbean caterpillar, *A. gemmatalis*, in southern Florida, two years after the release of the parasitoids, which persisted up to 3.5 years and spread over at least 9.1 km.

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