Biology and Morphology of *Chelonus* sp. nr. *curvimaculatus* (Hymenoptera: Braconidae) as a Parasitoid of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae)

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ABSTRACT A general description of the life stages of *Chelonus* sp. nr. *curvimaculatus*, an egg–larval parasitoid of pink bollworm, *Pectinophora gossypiella* (Saunders), is presented. Pink bollworms were reared in the laboratory on a wheat germ diet. At 29°C, parasitoid eggs (0.12–0.18 mm) begin to eclose ≈22 h after oviposition. Three instars occur in this species. The 1st instar is endoparasitic and ranged in length from 0.14 mm (neonate) to 1.25 mm (≈9 d old, end of the 1st stadium). The 2nd instar also is endoparasitic and is 1.89–3.04 mm long. This stadium lasts ≈2–3 d. The 3rd instar is an average of 3.82 mm long and is endoparasitic early in its development but becomes ectoparasitic toward the completion of its development. This stadium lasts ≈3 d. The pupal stage lasts ≈6–7 d. Adult parasitoids begin to emerge ≈21 d after oviposition. Parasitized and unparasitized pink bollworm larvae developed through 4 stadia. Larval head capsule widths, body lengths, and weights of parasitized pink bollworms are significantly smaller than those of unparasitized larvae during the 3rd and 4th stadia. Parasitized 4th-instar pink bollworms have a mean head width of 0.8687 mm, body length of 6.28 mm, and weight of 6.9 mg. Fourth-instar unparasitized pink bollworm measurements were 1.0743 mm, 9.31 mm, and 17.7 mg, respectively.

KEY WORDS *Chelonus* sp. nr. *curvimaculatus*, *Pectinophora gossypiella*, biological control, parasitoid

AFTER THE INTRODUCTION of the pink bollworm, *Pectinophora gossypiella* (Saunders), into the United States, repeated attempts to establish biological control agents from the 1930s to the 1950s failed (Noble and Hunt 1937; McGough and Noble 1955, 1957). Biological control agents, including exotic parasitoids from abroad, were collected (Legner and Medved 1979). None became established, but several of them reduced pink bollworm populations during the years of release.

Originally, 2 species of *Chelonus* were collected from Addis Ababa, Ethiopia, and a 3rd species was collected from Australia. All were referred to as *Chelonus* sp. nr. *curvimaculatus*. The only characteristic noted in the literature for separating the 2 Ethiopian species was that 1 had red femora (red-femur form) and the other had black (black-femur form) (Legner and Medved 1979). No distinguishing characteristics were given for the Australian species. Only 1 species (Ethiopian red-femur) has remained in culture; the other 2 (Ethiopian black-femur and Australian species) have perished. *C.* sp. nr. *curvimaculatus* (red-femur) has become an im-

portant research tool in recent years (Jones et al. 1986, Grossniklaus-Burgin and Lanzrein 1990), and is also the focus of the present research.

The biologies of several potentially economically important *Chelonus* species have been studied (Vance 1932, Jackson et al. 1978, Powers and Oatman 1984, Grossniklaus-Burgin et al. 1994). *Chelonus curvimaculatus* (Cameron) was first described in 1906 from specimens collected from Cape Town, South Africa (Cameron 1906). This parasitoid can attack several species of Lepidoptera in at least 4 families (Broodryk 1969). In South Africa, *C. curvimaculatus* parasitizes 2 important lepidopteran pests, *Loxostege frustalis* (Zeller) and *Phthorimaea operculella* (Zeller). A description of all life stages of *C. curvimaculatus* is given by Broodryk (1969). The biology of *C.* sp. nr. *curvimaculatus* (red-femur) remains vague (Legner and Thompson 1977).

Descriptions of the morphology and life cycle of a prospective biological control agent are essential for understanding its potential effectiveness. Because there are several *Chelonus* species commonly studied for biological control purposes, we provide a description to distinguish *C.* sp. nr. *curvimaculatus* (Ethiopian red-femur) from the others. Furthermore, we document its life cycle in relation to the host, pink bollworm, reared under laboratory conditions. The following 3 aspects of its biology are described: (1) general morphology of its life stages, (2) its development and growth, and (3)

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development and growth of parasitized and un-parasitized pink bollworm larvae. Finally, a method for rearing *C.* sp. nr. *curvimaculatus* on pink boll-worm larvae is presented, which may be useful when limited numbers of parasitoids are needed. Further research is underway to determine the ef-fectiveness of *C.* sp. nr. *curvimaculatus* under a variety of environmental conditions.

Materials and Methods

General Rearing Techniques. A *C.* sp. nr. *curvi-maculatus* (Ethiopian, red-femur) colony was maintained at the Maricopa Agricultural Center in Maricopa, AZ, using pink bollworm as the host. It was originally started from individuals provided in 1993 from a colony being maintained at the West-ern Cotton Research Laboratory, USDA–ARS, Phoenix, AZ.

The *C.* sp. nr. *curvimaculatus* colony at the Mari-copa Agricultural Center was reared in an insectary under $29 \pm 1°C$ and a photoperiod of 14:10 (L:D) h. The photoperiod was provided by 2 fluorescent light fixtures, each holding two 40-W cool white light bulbs (Philips F40CW). The relative humidity was not controlled.

Twenty to thirty female–male pairs of adult para-sitoids were housed in a 3.8-liter plastic rectangular container. The open end was enclosed with a cloth sleeve, allowing easy access. Nourishment was pro-vided by a thin layer of fresh honey on the top of the container. Water was provided by moist cotton dental wicks placed at the bottom of the container. Each day, a paper sheet containing ≈5,000 pink bollworm eggs (1–3 d old, supplied by USDA–APHIS, Phoenix, AZ) was placed in the chamber for 24 h. After the egg sheet was removed, it was cut into pieces containing ≈1,000 eggs each. The egg sheets were attached to sterile, wooden tongue depressors, and placed into diet containers. The tongue depressors held the egg sheets out of the diet, which prevented them from soaking up excess moisture and molding. This method also reduced the number of escaped larvae by ensuring that ne-onate larvae first came into contact with the diet.

The diet container consisted of a 0.9-liter paper ice cream container (WLMA, Newark, NJ) half-filled with shredded wheat germ diet (Bartlett and Wolf 1985). Immediately after the eggs hatched, the tongue depressors were removed to reduce the possibility of fungal contamination. Approximately 14 d after hatching, the pink bollworm larvae enter a prepupal wandering period. During this period, larvae usually chew openings through the diet con-tainer and crawl out. This is designated as *cutout*. Cutout larvae were collected and separated into 2 groups, parasitized and unparasitized. We used size to separate the larvae because at cutout, parasitized larvae are substantially smaller than unparasitized larvae. Approximately 400 parasitized larvae were put into a 3.8-liter rectangular plastic container, partially filled with styrofoam beads, which the

pink bollworm larvae used as a pupation substrate. Adult parasitoids were collected as they emerged and were then placed in oviposition containers. Females were allowed to mate for 24 h before being presented host eggs.

C. sp. nr. *curvimaculatus* **Morphology and Devel-opment.** An egg sheet containing ≈10,000 pink bollworm eggs (12 h old) was divided into 2 groups. One egg sheet was placed into a parasitoid ovipo-sition chamber with 40 adult *C.* sp. nr. *curvimacu-latus* pairs for 24 h. The other egg sheet, which was placed into a similar chamber, served as a control not exposed to parasitoid wasps. A sample of the parasitized eggs was collected for dissection at 8 h after exposure to parasitoid females. The remaining parasitized eggs were divided into 3 groups and placed in separate diet containers. The same pro-cedure was followed for the control group. To monitor further development after cutout, larvae were placed in polystyrene petri plates (100 by 20 mm) containing styrofoam beads. All rearing con-ditions were the same as described for the general colony.

The pink bollworm eggs collected at 8 h were divided into 4 groups. At varying intervals (8, 20, 30, and 48 h after exposure), 1 group of eggs was immediately dissected in a drop of all-purpose blue ink (Foxboro Company, Foxboro, MA) placed on a microscope slide. A glass cover slide was gently pressed down on top of the egg until it ruptured. Parasitoid eggs and larvae stained a darker color than did surrounding host tissues.

To study the growth of the larval parasitoid, co-horts of parasitized pink bollworm larvae were dis-sected daily. Several measurements were made of the parasitoid—width of the head at its widest point, length of the body from the tip of the head to the last abdominal segment not including the anal vesicle, and length of the anal vesicle. All measure-ments are presented as means with standard devi-ations. Measurements also were made of adult para-sitoid length, from the front of the head to the posterior tip of the abdomen.

General morphological observations of the exter-nal anatomy of the parasitoid's egg, larval, pupal, and adult stages were also made. Representative figures of the different life stages were drawn from photographs. Voucher adult specimens are held in the insect collection at the University of Arizona, Tucson, AZ.

Pink Bollworm Growth and Development. To study the effects of parasitism on pink bollworm larvae, 3 different groups were monitored. Groups 1 and 2 were larvae that hatched from eggs exposed to female parasitoids. However, not all host eggs were attacked, and parasitized and ostensibly un-parasitized larvae resulted from the same diet con-tainers. It was not possible to separate these 2 types of larvae at an early age without dissecting them. These groups were designated as EX-P (exposed and parasitized) and EX-U (exposed but unparasit-ized). The third group (control group) consisted of

individuals that were never exposed to parasitoids and were designated as NEX-U. These were reared separately from the previous 2 groups. The development of the pink bollworm larvae was monitored by measuring head capsule widths, body lengths, and weights of live larvae. They were then dissected to determine the presence or absence of parasitoid larvae.

Head capsule widths (widest point) were used to distinguish instars of parasitized and unparasitized pink bollworm larvae. The distribution of the head widths of parasitized and unparasitized larvae were plotted separately to establish a size range for each instar. For parasitized larvae, the division between host 3rd and 4th instars was determined by observing developmental characteristics for both the host and its parasitoid. If the host larva had a head width of 0.6698–0.7486 mm but had a body length characteristic of a 3rd instar, and its parasitoid was a 1st instar without a noticeable anal vesicle, then the host larva was considered a 3rd instar. If the host larva had a head width within the same range but had a body length characteristic of a 4th instar and its parasitoid was a 2nd or 3rd instar, then the host was considered a 4th instar.

Weights of pink bollworm larvae were determined from groups of larvae from diet containers of parasitized or unparasitized larvae. Single larvae during the 1st and 2nd stadia could not be weighed accurately with the available microbalance (Mettler AE 50 series). Therefore, daily measurements were based on a number of individuals ($n = 80$–480) weighed together. After the larvae reached the 3rd stadium ($\approx$day 6), they were weighed individually. Parasitized pink bollworm 1st and 2nd instar could not be distinguished from unparasitized larvae, so larval weights consisted of a single group of a mixed population designated EX-PU. All measurements are given as means with standard deviations.

The 3 pink bollworm populations, EX-P, EX-U, NEX-U, were compared using the Smirnov test for cumulative frequencies of unknown distributions (Conover 1980). The dependent variables—head capsule width, body length, and weight—were statistically analyzed within each instar. The T statistic used in the Smirnov test is equal to the greatest vertical distance between 2 distribution functions from 2 samples drawn from independent populations (Conover 1980). The T statistic was estimated by inspecting ogive plots of the cumulative frequencies for 2 independent samples.

The 0.95 quantile (critical value) was calculated by the following equation for large sample approximations (Conover 1980):

$$w(0.95) = 1.22 \text{ sqrt } ([m + n]/mn), \qquad (1)$$

where m and n are the sample sizes of the 2 contrasted populations. The 0.90 quantile was calculated similarly, but with a different scalar (i.e., 1.07). The null hypothesis that populations did not differ from one another was rejected if $T > w$.

Results

Biology of *C.* sp. nr. *curvimaculatus*. *Chelonus* sp. nr. *curvimaculatus* is an egg–larval parasitoid; the parasitoid egg is oviposited in a host egg and completes development in the host larva. After emerging from the pupation cell, the female parasitoid is ready to oviposit whether she has mated or not. Upon detecting a host egg, she antennates with the tips of her antenna several times before she oviposits. If the host egg is acceptable, oviposition lasts $\approx$20–40 s, one egg being laid during an ovipositional bout. Superparasitized host eggs were found in many instances; however, only 1 parasitoid could complete development in a host. The cause of death of the supernumerary parasitoids was undetermined.

There are 3 parasitoid instars. First instars begin to hatch $\approx$22 h after oviposition with complete eclosion by $\approx$30 h. They are endoparasitic and constitute the majority of the larval period ($\approx$9 d). It is believed that the parasitoid larvae are primarily feeding on the host hemolymph during this period. The 1st instar was observed to molt into the 2nd instar only after the host larva had entered its final instar (4th instar).

The 2nd instar is endoparasitic and is thought to feed primarily on the host's hemolymph. The exuviae of the 1st instar often is attached to the last abdominal segment. The parasitoid's 2nd stadium lasts $\approx$2–3 d.

The 3rd instar is endoparasitic early in its development and ectoparasitic later. This stadium lasts $\approx$3 d. The early 3rd instar lives and feeds within the host until it chews a hole through the thoracic region of the host and emerges. The parasitoid larva then bends around and devours its host, beginning at the posterior end. The last few abdominal segments of the parasitoid remain in the host until this feeding is complete. The parasitoid consumes all of the host except for the head capsule and body cuticle. After it devours its host, the parasitoid spins its cocoon and pupates in the cocoon of the host, where it remains for 6–7 d, after which the adult emerges. If a pink bollworm puparium is not present, the larva usually fails to pupate and will die. The parasitoid develops from an egg to an adult in $\approx$21 d.

Description of *C.* sp. nr *curvimaculatus* Life Stages. *Egg.* The egg is white, hymenopteriform with a cylindrical arcuate shape. Both ends are round, and the cephalic end is slightly larger than the caudal end (Fig. 1A). The length is 0.12–0.18 mm ($n = 27$).

Larvae. The neonate larva is translucent white and has a squarish head, flattened dorsoventrally, and has 7 visible body segments; these segments are all of nearly the same size, except the last is slightly longer and pointed posteriorly. The dominant features on the head are 2 laterally attached, heavily sclerotized, sickle–shaped mandibles; 2 labral processes; and the labium (Fig. 1B); the last 3 are
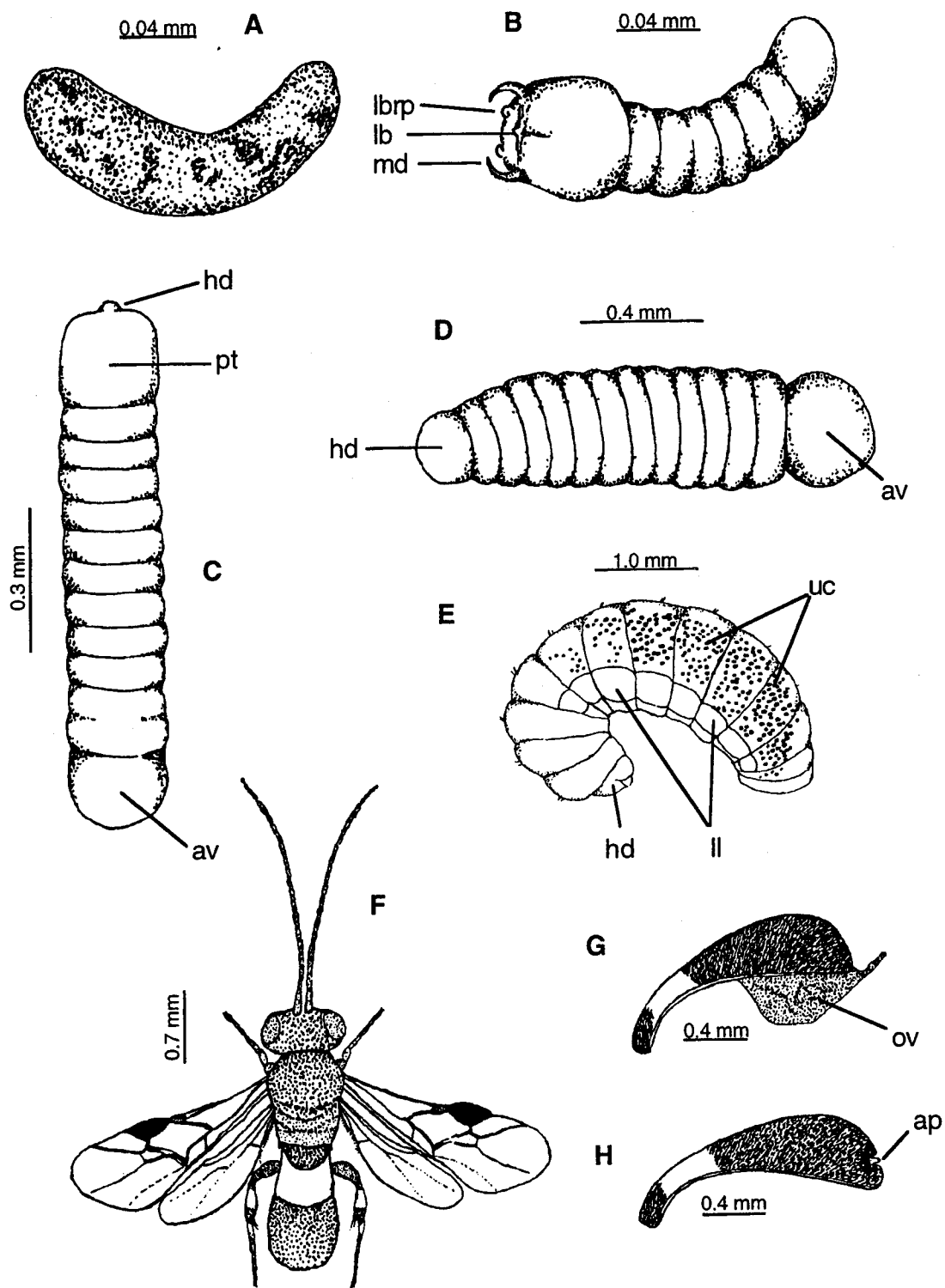
Fig. 1.   Different life stages of *C.* sp. nr. *curvimaculatus*. (A) Egg. (B) Neonate, ventral view. (C) Late 1st instar, dorsal view. (D) Second instar, dorsal view. (E) Third instar, lateral view. (F) Adult male. (G) Lateral view of female abdomen with ovipositor. (H) Lateral view of male abdomen with anal pit. ap, anal pit; av, anal vesicle; hd, head; lb, labium; lbrp, labral process; ll, lateral lobes; md, mandible; ov, ovipositor; pt, prothorax; uc, urate cells.
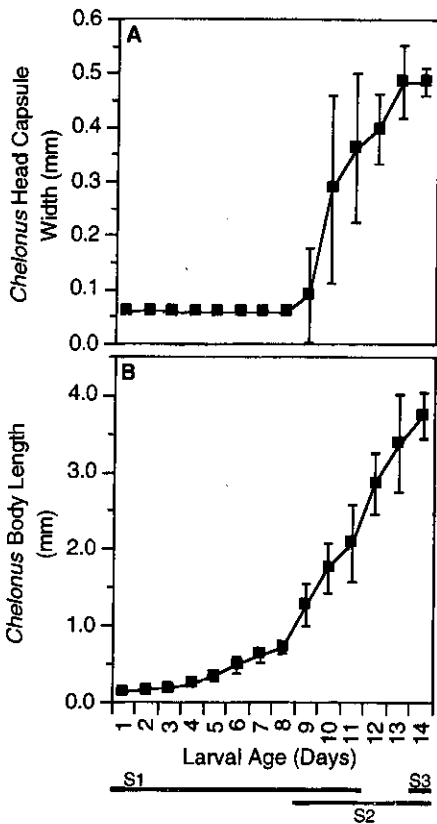
Fig. 2. Daily head capsule widths (A) and body lengths (B) of *C.* sp. nr. *curvimaculatus* larvae. Data are presented as means ± SD. *C.* sp. nr. *curvimaculatus* stadia are represented as bars below the x-axis. Days 1 and 14 ($n = 32$); days 2–13 ($n = 15$–25).

ventral. The dorsum is sparingly covered with small spines. Neonate larvae are usually surrounded by serosal cells.

The late 1st instar is characterized by 12 visible body segments and an enlarged anal vesicle (Fig. 1C). The 1st segment posterior to the head capsule is 2–3 times longer than any of the following segments. The anal vesicle begins to appear around day 6 of development. The body changes from the translucence of the neonate larva to a more opaque white.

The neonate larva's body length and head width are 0.14 ± 0.01 mm and 0.06 ± 0.00 mm ($n = 32$), respectively. A fully developed 1st instar is 1.25 ± 0.27 mm ($n = 23$) long and its head width is the same as that of the neonate larvae (Fig. 2; Table 1).

The 2nd instar is creamy white (Fig. 1D); its head is larger than that of the 1st instar, followed by 13 visible body segments and a large anal vesicle which is typically indented posterodorsally. The head and mouthparts are unsclerotized. This instar also has antennal disks on the dorsal surface of the head, urate cells (which appear as white dots through the cuticle) within the abdominal segments, and the beginning of a tracheal system.

A young 2nd instar's body length and head width are 1.89 ± 0.26 mm and 0.39 ± 0.05 mm ($n = 16$), respectively. An older 2nd instar is 3.04 ± 0.46 mm long and its head is 0.44 ± 0.08 mm ($n = 36$) wide (Fig. 2; Table 1).

The 3rd (final) instar (Fig. 1E) has an average body length of 3.82 ± 0.25 mm and an average head width of 0.49 ± 0.03 mm ($n = 34$) (Fig. 2; Table 1). The early 3rd instar is endoparasitic and can be distinguished from the 2nd instar by its slightly sclerotized mouthparts and the development of lateral lobes on the abdominal segments. The tracheal system has more visible branches, and the urate cells appear whiter and larger. As development of the 3rd instar continues, the anal vesicle is lost.

The fully developed 3rd instar parasitoid is characterized by fully sclerotized mouthparts as well as very enlarged lateral lobes on segments 5 through 12. The creamy white body of the early 3rd instar becomes more pinkish. Short spines occur on the dorsal surface of the body; these were less noticeable in the early 3rd instar.

Pupa. After the 3rd-instar parasitoid spins its silver-white cocoon it becomes stationary. During this period, the larva shrinks slightly, and the abdomen becomes wider. The gut contents become a homogenous mass of material. This stationary period of the larva is considered the prepupa.

The pupa is exarate. Its color changes with age, at first pale and then darkening. Just before ecdysis, the pharate adult is visible and the pupa becomes black.

Adult. The adults (Fig. 1F) have a black head and body except for a large white band at the anterior end of the abdomen. The scape and pedicel of the antennae are yellowish brown, and the flagellum is yellowish brown proximally, gradually darkening to black distally. The prothoracic legs are yellowish brown; the last tarsomere is black. The mesothoracic legs are like the prothoracic, except for a dark band on the femora; this is slightly darker in females. The femora of the metathoracic legs are dark with a yellowish brown anterior; tibiae are yellowish brown medially and darker both distally and

Table 1. Mean ± SD head capsule width, body length, and anal vesicle length for the *C.* sp nr. *curvimaculatus* larval instars

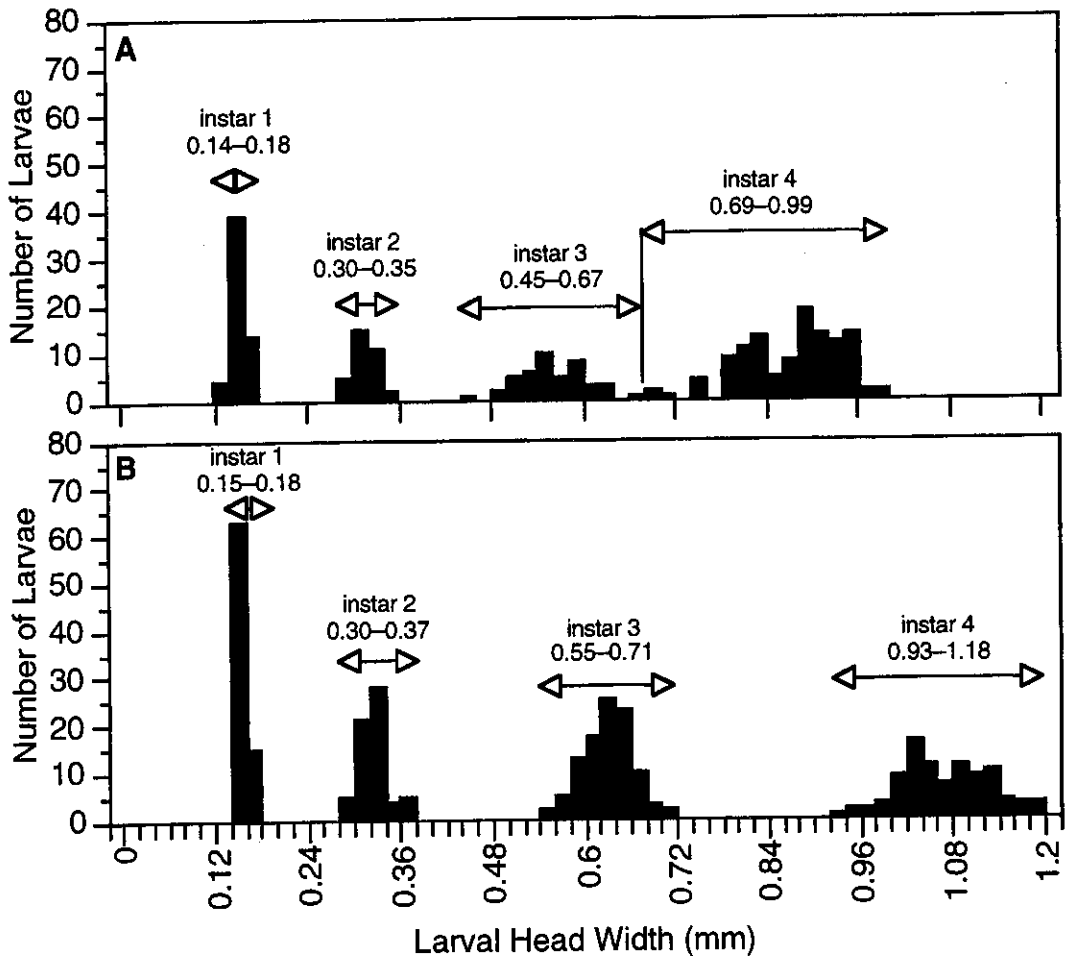| Measurement	1st instar ($n = 199$)		2nd instar ($n = 70$)		3rd instar ($n = 34$)	
	Mean, mm	Min.–Max	Mean, mm	Min.–Max	Mean, mm	Min.–Max
Head width	0.0594 ± 0.0014	0.0552–0.0690	0.4207 ± 0.0671	0.2758–0.5910	0.4890 ± 0.0276	0.4334–0.5516
Body length	0.4947 ± 0.4058	0.1005–1.6548	2.5599 ± 0.6464	1.4775–3.9984	3.8243 ± 0.2457	3.1360–4.3120
Anal vesicle	0.0508 ± 0.0912	0.0000–0.3152	0.3820 ± 0.0706	0.2352–0.5880	0.1268 ± 0.1683	0.0000–0.4704

Fig. 3. Distribution of head capsule widths of parasitized (A) and unparasitized (B) pink bollworm larvae. Measurement interval is 0.02 mm.

proximally; tarsi are similar to the pro- and meso-thoracic tarsi.

Several differences occur between the 2 sexes. Male antennae have 22-25 segments; those of the female have 16 segments. The body length of males is 3.19 ± 0.17 mm ($n = 40$); of females 3.43 ± 0.14 mm ($n = 40$). Lastly, the females have a small ovipositor (Fig. 1G), and the males have an oblong pit at the apex of the abdomen (Fig. 1H).

Pink Bollworm Growth and Development. The 4 instars of the pink bollworm were distinguished based on the distribution of their head capsule widths (Fig. 3 A and B); these groups were clear for unparasitized larvae (Fig. 3B), but the distribution of these widths in parasitized larvae was more variable (Fig. 3A). Although only 4 instars were recognized, the distribution shown in Fig. 3A might indicate that some individuals were preparing to undergo a supernumerary molt; those larvae had head capsule widths in the range of 0.6698–0.7486 mm. However, the control population did not con-

tain such individuals. Individuals that pass through a supernumerary molt have slightly larger head widths in their final stadium than larvae with only 4 instars (Watson and Johnson 1974). Such individuals are not present in either the parasitized or unparasitized pink bollworm populations in this study (Fig. 3).

The 1-sided Smirnov test was used to compare differences in the pink bollworm populations (Table 2). Fig. 4 is an example of how the T statistic (Smirnov test) was determined using the 4th instar weight comparison between EX-P and NEX-U. The greatest vertical distance between the cumulative frequencies for the comparison EX-P/NEX-U, gave the T value 0.930 ($P \le 0.05$).

All 3 larval groups grew at about the same rate until the 3rd stadium (Table 3). During the 3rd and 4th stadia, the 3 larval cohorts tended to show different growth patterns (Fig. 5). The EX-P larvae were the smallest and the NEX-U larvae were the largest. Generally, all 3rd and 4th instars in the 3

Table 2. Smirnov's test statistics, *T*, for pink bollworm head capsule width, body length, and weight frequency distributions

Comparison[a]	Instar	n	Head capsule width	Body length	Wt frequency
NEX-U/EX-P	1	57/57	0.090	0.150	—
EX-P/EX-U	1	57/21	0.120	0.235	—
NEX-U/EX-U	1	57/21	0.038	0.140	—
NEX-U/EX-P	2	36/33	0.185	0.190	—
EX-P/EX-U	2	33/27	0.198	0.290	—
NEX-U/EX-U	2	36/27	0.140	0.345**	—
NEX-U/EX-P	3	49/44	0.680**	0.485**	0.880**[a]
EX-P/EX-U	3	44/51	0.605**	0.380**	0.480**[b]
NEX-U/EX-U	3	49/51	0.245*	0.220*	0.510**[c]
NEX-U/EX-P	4	74/116	0.975**	0.960**	0.930**[d]
EX-P/EX-U	4	116/17	0.845**	0.560**	0.560**[e]
NEX-U/EX-U	4	74/17	0.495**	0.670**	0.735**

NEX-U, not exposed and unparasitized; EX-P, exposed and parasitized; EX-U, exposed and unparasitized. *, $P < 0.10$; **, $P < 0.05$.
[a] $n = 19/15$.
[b] $n = 15/35$.
[c] $n = 19/35$.
[d] $n = 74/115$.
[e] $n = 115/17$.

cohorts were significantly different ($P < 0.05$) from one another in the 3 parameters measured (Table 2). First- and 2nd-instar weights could not be statistically analyzed because weights were of samples drawn from a mixed population of parasitized and unparasitized larvae.

Unparasitized larvae appear to pupate sooner than parasitized larvae (Fig. 5). However, this is an artifact caused by the sampling period for the 2 different types of larvae. Unparasitized larvae were monitored only until cutout, whereas parasitized larvae were monitored until the parasitoid emerged and consumed its host. Normally, parasitized larvae start to cutout 1–2 d before unparasitized larvae.

Lastly, when observing normal unparasitized male larvae, the developing testes can be seen through the dorsal cuticle as 2 light brown small,

Fig. 4. Cumulative frequency of weights for parasitized (EX-P) and unparasitized (NEX-U) pink bollworm 4th instars. T represents the greatest vertical distance between the cumulative frequencies for EX-P and NEX-U. Measurement interval is 0.8 mg.

oval structures. Upon dissections of many parasitized larvae, no testes were found, suggesting sterility of the larvae before their death.

Discussion

Chelonus species have a unique and complex association with their host. The parasitoid egg is laid in the host egg, but development of the larva occurs in the developing host larva. In addition, *Chelonus* have some control over the development of their host (Jones et al. 1986). Serosal cells (teratocytes), which surround neonate *Chelonus*, are believed to play a significant role in the regulation of the endocrine system of the host (Dahlman and Vinson 1993).

In the *C.* sp. nr. *curvimaculatus-Trichoplusia ni* system (Jones et al. 1981, 1986), host larvae that are parasitized by *C.* sp. nr. *curvimaculatus* initiate precocious metamorphosis in the penultimate instar. We found that both parasitized and unparasitized larvae appear to develop through 4 stadia, suggesting that parasitized larvae do not initiate metamorphosis in the penultimate instar. This difference may be a result of the 2 different host systems under study.

In the *C.* sp. nr. *curvimaculatus-T. ni* system, Jones et al. (1981, 1986) found that both parasitized and pseudoparasitized *T. ni* larvae remain in a state of developmental arrest in the prepupal stage upon precocious metamorphosis in the penultimate instar. In the current study, on several occasions, pink bollworm larvae failed to pupate at the normal time and upon dissection, no parasitoid larvae were found. It is not clear if these larvae were pseudoparasitized and were in a state of arrested development, experienced delayed development, or were in diapause.

There are some contradictory reports on the development of the 1st-instar head of *Chelonus* species. The segment just posterior to the sclerotized
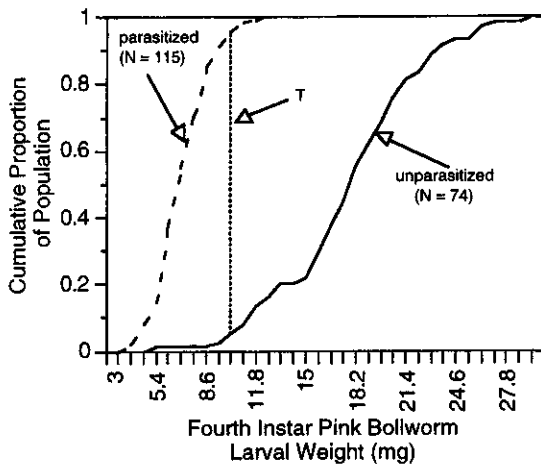
Table 3. Pink bollworm larval head capsule width, body length, and weight measurements, mean ± SD

Cohort	Instar	n	Length, mm	Min./Max., mm	Head width, mm	Min./Max mm	Wt, g	Min./Max., g
NEX-U	1	57	1.04 ± 0.21	0.77/ 1.64	0.1593 ± 0.0050	0.1478/0.1773	—	—
EX-P	1	57	1.08 ± 0.30	0.59/ 1.83	0.1587 ± 0.0083	0.1379/0.1773	—	—
EX-U	1	21	1.00 ± 0.17	0.73/ 1.46	0.1600 ± 0.0043	0.1576/0.1714	—	—
NEX-U	2	36	2.14 ± 0.55	1.24/ 3.41	0.3270 ± 0.0161	0.2955/0.3743	—	—
EX-P	2	33	2.13 ± 0.43	1.38/ 3.02	0.3203 ± 0.0158	0.2955/0.3546	—	—
EX-U	2	27	2.39 ± 0.47	1.67/ 3.41	0.3300 ± 0.0209	0.2955/0.3704	—	—
NEX-U	3	49	4.88 ± 1.07	2.90/ 6.54	0.6340 ± 0.0298	0.5516/0.6895	0.0052 ± 0.0011[a]	0.0038/0.0070
EX-P	3	44	3.89 ± 0.80	2.47/ 5.74	0.5603 ± 0.0432	0.4531/0.6698	0.0029 ± 0.0007[b]	0.0018/0.0043
EX-U	3	51	4.61 ± 0.97	2.67/ 6.54	0.6240 ± 0.0342	0.5516/0.7092	0.0039 ± 0.0011[c]	0.0020/0.0063
NEX-U	4	74	9.31 ± 0.96	6.06/11.08	1.0743 ± 0.0538	0.9259/1.1820	0.0177 ± 0.0046[d]	0.0053/0.0288
EX-P	4	116	6.28 ± 0.63	4.04/ 7.73	0.8687 ± 0.0636	0.6895/0.9850	0.0069 ± 0.0017[e]	0.0035/0.0119
EX-U	4	17	7.64 ± 0.93	6.14/ 9.09	1.0151 ± 0.0457	0.9456/1.1032	0.0106 ± 0.0031	0.0059/0.0166

NEX-U, unparasitized; EX-P, parasitized; EX-U, exposed to potential parasitism but unparasitized.
[a] $n = 19$.
[b] $n = 15$.
[c] $n = 35$.
[d] $n = 74$.
[e] $n = 115$.

portion of the head contains the developing brain and greatly expands during development, which may indicate that the head is composed of a sclerotized and nonsclerotized portion (Broodryk 1969, Grossniklaus-Burgin et al. 1994). Vance (1932) and Jackson et al. (1978) suggested that the enlarged segment is the prothorax. This seems likely, because the labium in *C.* sp. nr. *curvimaculatus* is attached to the sclerotized portion of the head, thus suggesting that the actual head does not include the nonsclerotized portion. In insects, the labium is derived from the most posterior segment of the head (Snodgrass 1935).

The 2nd instar of *C.* sp. nr. *curvimaculatus* has a nonsclerotized head, which expands during development, and nonsclerotized mouthparts like those of other *Chelonus* species (Broodryk 1969, Jackson et al. 1978, Powers and Oatman 1984). *Chelonus inanitus* (L.), however, lacks mandibles in this instar (Grossniklaus-Burgin et al. 1994). It is not clear why 2nd-instar *Chelonus* lack sclerotized mouthparts; perhaps there is little need for them. Second-instar parasitoids do not appear to cause much damage to the internal organs of the host (unpublished data) and therefore probably feed mainly on host hemolymph.

The growth of parasitized pink bollworm larvae appears to be similar to that of unparasitized larvae, during their 1st and 2nd stadia. At the beginning of the 3rd stadium, parasitized larvae begin to show signs of reduced growth, indicating that the effects due to parasitism actually begin in the 2nd stadium of the host. By the end of the 4th stadium of the host, parasitized larvae are substantially smaller than unparasitized larvae.

Fourth-instar EX-U are significantly smaller than NEX-U larvae. This discrepancy between the 2 larval groups could not be definitively explained. A portion of the EX-U larvae may have been pseudoparasitized, and therefore may be smaller than unparasitized larvae (Jones et al. 1986). Container
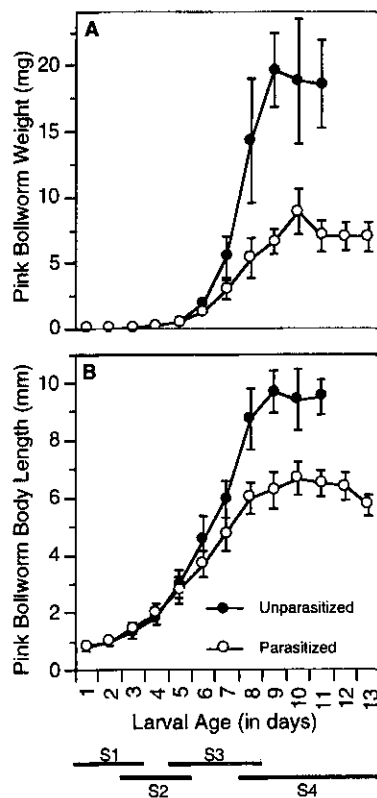

Fig. 5. Daily body length (A) and weight (B) measurements for parasitized (EX-P) and unparasitized (NEX-U) pink bollworm larvae. All data are given as means ± SD. Temporal ranges of the 4 stadia of EX-P and NEX-U larvae are represented by the bars below the x-axis. Determinations of body length were based on the following sample sizes (n): NEX-U, $n = 20$ for all days except day 6 $(n = 23)$ and day 11 $(n = 11)$; for EX-P, day 1–12 $(n = 15$–$25)$, day 13 $(n = 11)$. Weight measurements for parasitized larvae (days 1–6) were a mixed population of parasitized and unparasitized larvae (EX-UP). For day 1; $n = 479$ (NEX-U), $n = 421$ (EX-UP): days 2–6; $n = 79$–148 for NEX-U and EX-UP: for days 7–13; $n = 13$–20 (NEX-U), $n = 11$–25 (EX-P).

effects could not be ruled out because of the lack of true replicates; however, except for being exposed to potential parasitism, the eggs, and consequently the larvae, from both groups were exposed to the same conditions. Experimental error may have been involved if smaller larvae were unknowingly being selected out of the parasitized diet containers, and larger larvae were being selected out of unparasitized containers, for examination and dissection.

In conclusion, the relationship between *C.* sp. nr. *curvimaculatus* and pink bollworm is an intricate association in which the parasitoid regulates its own development and also has some control over the development of its host. Parasitized larvae are smaller than unparasitized larvae and usually start cutting out 2 d earlier. They may, therefore, consume less food than unparasitized larvae. This is important to biological control. Not only will parasitized larvae die, but they also may cause less damage to cotton during development.

Acknowledgments

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