Effect of the Parasite *Copidosoma truncatellum*[1] on Development of Its Host *Trichoplusia ni*[2,3]

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ABSTRACT

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Parasitism of larvae of *Trichoplusia ni* (Hübner)by *Copidosoma truncatellum* (Dalman) caused 33 to 58% of the *T. ni* larvae to undergo a supernumerary molt, regardless of whether the host was from a normal culture which pupated at the end of the 5th or 6th instar. Parasitized larvae which did not enter a supernumerary instar gained weight significantly faster and had greater head capsule sizes than unparasitized larvae. Parasitized larvae which entered a supernumerary instar gained weight more slowly and possessed smaller head capsules than unparasitized larvae, except for the supernumerary instar, which had a significantly greater final weight and head capsule size than achieved by unparasitized larvae.

Copidosoma truncatellum (Dalman) is a polyembryonic egg-larval parasite of *Trichoplusia ni* (Hübner). Recent research addresses the potential of *C. truncatellum* as a mass-release agent for *T. ni* control (Stoner and Weeks 1974, 1976, Hunter and Stoner 1975). These studies indicate that several physiological characteristics may prevent *C. truncatellum* from being a good mass-release agent. One limiting characteristic was the reported induction of 2 additional days host feeding during the last instar (Hunter and Stoner 1975). Neither this nor other limiting characteristics, however, prevents natural populations of *C. truncatellum* from being, on occasion, an effective mortality factor (Stoner and Weeks 1976).

When using life table analysis to evaluate the impact of natural enemies, the developmental times of *T. ni* stage are needed for computation of host population density. The extended larval stage of *T. ni*, as a result of parasitization by *C. truncatellum*, makes such computations more complex. Though Hunter and Stoner (1975) implied that only the duration of the last instar of parasitized *T. ni* was affected, Jones and Van Steenwyk (unpublished data) had difficulty in classifying the larval stages of *T. ni* to the proper instar because of abnormal head capsule sizes. The *T. ni* field population which the latter studied was heavily parasitized (60%) by *C. truncatellum*. The present study was conducted to quantify the effect of *C. truncatellum* on the duration and growth of *T. ni* instars.

Materials and Methods

Two separate cultures of *T. ni* in which larvae usually underwent a constant number of instars (five or six) were reared under standardized conditions of 14:10 L:D, 28° C, on a bean diet (Shorey and Hale 1965, Sparks et al. 1979). The *C. truncatellum* culture was obtained by rearing adults from parasitized *T. ni* collected from celery at Irvine, Calif., and was maintained under conditions similar to the *T. ni* culture.

Sheets of paper on which *T. ni* eggs had been oviposited were exposed to *C. truncatellum*. The paper was removed, and sections were stapled under the lid of a 177-ml cup containing *T. ni* diet. On hatching, individual parasitized and unparasitized larvae were each placed into separate 30-ml cups containing a similar amount of diet. Individually reared larvae were observed daily throughout development.

Between 1900 and 2200 h each day (day begins at lights off), larvae were weighed to the nearest 0.01 mg. The stage of development (instar, occurrence of spinning, etc.) was recorded daily, and the width of shed head capsules was measured at the widest point. Some head capsules could not be located in the frass and diet and, with a few larvae, it could not be definitely determined on which day a larval-larval molt had occurred, since a few larvae molted before or after 1900 to 2200 h. For computations concerning instar duration, the average of the two possible durations was used (e.g., [3 days + 4 days]/2 = 3.5 days for that larva). Measurements were terminated when larvae began spinning a cocoon for pupation. Only larvae surviving to cocoon spinning were used for analysis, since some larvae died before this event.

The efficiency of food conversion into biomass by parasitized and unparasitized larvae was compared by taking 16 last (6th) instars from each group and weighing them on the day of the penultimate apolysis and again on the day of cessation of feeding (day of cocoon spinning) as well. Diet cups were also weighed after the feces had been removed. Ten control diet cups containing no larvae were also weighed at the same time to assess loss of diet weight due to evaporation.

The effect of nutrient deficiency on normal metamorphosis of the last instar was tested by placing 4th (penultimate) instars during head capsule slippage to the 5th (last) instar into individual cups containing no diet. Larvae were starved for 1, 2, or 3 days, then placed in individual cups containing diet and their development observed. Twenty larvae

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were tested for each of the three starvation regimes. In another test, 4th instars were selected during head capsule slippage to the last instar and placed in individual diet cups. The larvae were allowed to feed for 1, 2, or 3 days before being removed from food, and their development was observed. Twenty larvae were tested for each feeding regimen.

Comparison of means involving only two groups was done with a two-tailed t test. When multiple comparisons involving more than two groups was required, Duncan's new multiple range test, with Kramer's modification for unequal sample sizes (KDNMR),was used. For both tests, an α level of 0.05 was selected.

Results

Larvae Normally Undergoing Six Larval Instars

The duration of each instar in both parasitized and unparasitized larvae is given in Table 1. Both groups required a similar amount of time from egg hatch until the molt to the 6th instar (15.0 to 15.6 days).Unparasitized larvae began cocoon spinning after 5.5 days into the 6th instar, and none underwent additional molts. However, only 64% of the parasitized larvae spun cocoons in the 6th instar; the remainder molted to a supernumerary 7th instar. Parasitized larvae spinning cocoons as either 6th or 7th instars spun cocoons after about 5.5 days into the last instar, like unparasitized larvae.

The total time from egg hatch to cessation of feeding and initiation of spinning was about 21 days in both parasitized larvae spinning as 6th instars and unparasitized larvae. Parasitized larvae molting to 7th instars took 24 days to reach cocoon spinning because of 3.4 days spent feeding in the 6th instar before the supernumerary molt. After the supernumerary molt, the larvae fed for 5.2 days. Thus, the overall duration of feeding by parasitized larvae undergoing a supernumerary molt was greater than that of unparasitized larvae, but this effect was not due to extended feeding during the last instar.

Hunter and Stoner (1975) found that parasitized and unparasitized larvae are separable on the basis of differences in weight gain, with parasitized larvae gaining weight more slowly. Our data (Table 2) indicate a more complex situation. Parasitized 4th and 5th instars destined to spin as 6th instars gained more weight than unparasitized larvae. The weight gain of those spinning as 7th instars was lower than normal. The final average maximum weights of the two parasitized groups were not significantly different (303 and 336 mg), and each was greater than that of unparasitized larvae by ca. 20 to 30%.

Head capsule widths of parasitized and unparasitized larvae were the same until the 5th instar (Table 3). Then, as with trends in body weight, parasitized larvae spinning as 6th instars developed larger head capsules than unparasitized larvae, whereas larvae spinning as 7th instars had capsule widths significantly smaller than unparasitized larvae. The head capsule width of last instars of either group of par-

Table 1.—Duration (days) of larval stages of *C. truncatellum*-parasitized *T. ni*

T. ni	Sample size and duration (X days ± SD) of the following larval stages[a]													
	1		2		3		4		5		6		7	
	n		*n*		*n*		*n*		*n*		*n*		*n*	
Normal	17	a4.0 ± 0.6	12	a2.8 ± 1.0	20	a2.4 ± 0.5	22	a3.0 ± 1.1	25	a3.5 ± 1.9	25	a5.5 ± 1.3	—	—
Parasitized[b]	28	a3.8 ± 1.4	14	a2.3 ± 0.5	30	a2.7 ± 0.8	30	a3.0 ± 1.6	30	a3.7 ± 0.8	30	a5.5 ± 1.0	—	—
Parasitized[c]	31	a3.9 ± 1.5	10	a2.3 ± 0.5	13	a2.6 ± 1.1	13	a2.7 ± 0.7	13	a3.6 ± 1.5	13	b3.4 ± 0.7	13	5.2 ± 1.0

[a] Means preceded by different letters are significantly different (P < 0.05) by KDNMR. P value for F statistic of the group where differences were found was < 0.05.
[b] Spun cocoons as 6th instar.
[c] Spun cocoons as 7th instar.

Table 2.—Weight (mg) of normal and *C. truncatellum*-parasitized *T. ni* larvae undergoing head capsule slippage (HCS) during the molt to the next instar

T. ni	Sample size and wt ($\bar{x}$ mg ± SD) at HCS of the following instars:												
	n	2nd	*n*	3rd	*n*	4th	*n*	5th	*n*	6th	*n*	Avg. wt final instar
Normal[a] (spin as 6th instar)	19	a2.3 ± 0.6	26	ab7.4 ± 3.0	26	b21.7 ± 8.9	23	b77.5 ± 22	—	—	18	a253.1 ± 42.8
Parasitized[b] (spin as 6th instar)	52	a2.0 ± 0.5	43	a8.3 ± 2.5	43	a27.3 ± 5.2	42	a97.3 ± 18.5	—	—	40	b303.2 ± 57.9
Parasitized[c] (spin as 7th instar)	11	a2.1 ± 0.5	15	b6.2 ± 2.7	18	b18.1 ± 6.4	19	c46.2 ± 10.3	19	128.9 ± 26.4	19	b336.0 ± 85.5

[a] Means preceded by different letters are significantly different ($\alpha = 0.05$) by KDNMR. *P* values for F statistic of groups where differences were found were (left to right): < 0.05, < 0.005, < 0.005, and < 0.005.
[b] Spin cocoons as 6th instar.
[c] Spin cocoons as 7th instar.

Table 3.—Head capsule widths of normal and *C. truncatellum*-parasitized *T. ni.* larvae

T. ni	Sample size and head capsule widths ($\bar{x}$ mm ± SD) of various instars													
	n	1	*n*	2	*n*	3	*n*	4	*n*	5	*n*	6	*n*	7
Normal[a]	10	a0.29 ± 0.010	8	a0.45 ± 0.03	18	a0.69 ± 0.08	23	a1.01 ± 0.13	31	b1.40 ± 0.13	12	b1.90 ± 0.08	—	—
Parasitized[b]	10	a0.29 ± 0.006	24	a0.45 ± 0.05	21	a0.70 ± 0.06	31	a1.03 ± 0.10	24	a1.52 ± 0.13	24	a2.04 ± 0.12	—	—
Parasitized[c]	10	a0.29 ± 0.007	5	a0.45 ± 0.03	13	a0.69 ± 0.10	13	a0.97 ± 0.11	13	b1.35 ± 0.16	11	c1.73 ± 0.18	13	2.20 ± 0.09

Means preceded by different letters are not significantly different ($\alpha = 0.05$) by KDNMR. *P* values for F statistic of groups where differences were found were (left to right): < 0.005 and < 0.005.
[b] Spin cocoons as 6th instar.
[c] Spin cocoons as 7th instar.

asitized larvae was larger than that of unparasitized larvae (1.90 mm). The final head capsule size for parasitized 7th instars (2.20 mm) was greater than parasitized 6th instars (2.04 mm).

The efficiency of conversion of food consumed to biomass (change in weight per food consumed times 100, Waldbauer [1968]) was 16.4 ± 4.04% and 16.9 ± 3.9% in parasitized and unparasitized larvae, respectively, and was not significantly different (t, $P > 0.05$) in the two groups. The parasitized larvae consumed significantly more diet (1.43 ± 0.30 g) than the unparasitized larvae (1.01 ± 0.12 g) (t, $P < 0.05$).

Larvae Normally Undergoing Six Larval Instars

Since previous workers studying *C. truncatellum* had used cultures of *T. ni* which normally underwent five larval instars, parasitized hosts form such a culture were observed for comparative purposes against the results obtained from the 6-instar culture. Of 33 parasitized larvae, 58% underwent a supernumerary molt to a 6th instar, whereas none of 100 controls did. Both parasitized and unparasitized larvae developed at similar rates, all having reached the 5th instar by 11 days after hatching. However, by day 15 all unparasitized larvae had spun, whereas only 42% of the parasitized larvae had spun. The comparative weight gain of unparasitized larvae, parasitized larvae spinning in the 5th instar, and parasitized larvae spinning as supernumerary 6th instars, followed the same trend as in Table 2, and the maximum weights were 325.0 ± 33.7, 430.4 ± 81.8, and 410.4 ± 87.9 mg, respectively. Thus, parasitized larvae had significantly (KDNMR, 0.05) greater maximum weight (ca. 30%) than unparasitized larvae.

Effect of Starvation on Metamorphosis

Whether larvae were treated normally, starved for the first 1, 2 or 3 days of the last larval stage before feeding, or starved after feeding for the first 1, 2, or 3 days of the last instar, all insects either died or pupated. In no case was a supernumerary instar observed in either larvae which normally pupate in the 5th instar or the 6th instar.

Discussion

Since the parasitized and unparasitized larvae were reared under the same conditions of food, temperature, photoperiod, and size of rearing containers, and supernumerary molts were observed only in the parasitized larvae, it is concluded that the supernumerary molting was a consequence of parasitism. The supernumerary molting was observed both in hosts from a culture normally undergoing five instars (58% supernumerary molts) and from a culture normally undergoing six instars (33% supernumerary molts). The induction of an extra instar in host *T. ni* by *C. truncatellum* was not reported by Hunter and Stoner (1975). King and Atkinson (1928) reported, in passing, that some *Euxoa ochrogaster* (Guenneé) (Noctuidae) parasitized by *Copidosoma bakeri* Howard underwent a supernumerary molt.

Hunter and Stoner (1975) observed an additional 2 days of feeding in *C. truncatellum* parasitized larvae and 48% of parasitized larvae had spun cocoons by the time 93% of unparasitized larvae had. They attributed this delay in spinning to an increased duration of the last instar of parasitized larvae. Our data indicate the 2 additional days of feeding were probably due to the interposition of a short, penultimate instar before a supernumerary instar.

Hunter and Stoner (1975) used the method of Waldbauer (1968) to estimate and compare the efficiency of food conversion of unparasitized and parasitized *T. ni* larvae. They stated that parasitized larvae were more efficient but did not provide values of efficiency of conversion or a statistical analysis. We found no significant difference between the conversion efficiencies of unparasitized and parasitized larvae during their 6th (last) instar. Though the data format of Hunter and Stoner (1975) precludes complete analysis, division of the reported weight gained by the amount of food consumed from 7 days post-egg hatch to the first day that any larvae ceased feeding for pupation (days 11 and 13 in unparasitized and parasitized larvae, respectively) results in apparently similar efficiencies.

Our data illustrate the complexities that *C. truncatellum* introduces into *T. ni* life table construction. First, many parasitized larvae have above- or below-normal head capsule sizes and body weights. These data explain why Jones and Van Steenwyk had difficulty in estimating the instar of some field collected *T. ni* larvae. The second complexity, first observed by Howard (1908), concerns the possible overestimation of larval density and percent parasitism as a consequence of the extended duration of the larval stage in many parasitized individuals. The penultimate and last instars are often combined during field sampling ("large" larvae). Parasitism by *C. truncatellum* would cause overestimation of the density of "large" larvae. It would also cause underestimation of the difference between the densities of "medium" and "large" larvae, that is, the number of "medium" larvae which had disappeared due to predation.

The occurrence of a supernumerary instar in the host does not demonstrate active, deliberate host regulation by the parasite as opposed to indirect disturbances of host physiology. However, several models for indirect induction of a supernumerary instar in parasitized *T. ni* may be rejected.

It was found by Decker and Maddox (1971) for *Simyra henrici* (Grote) and by Nijhout (1975) for *Manduca sexta* (L.) that pupation will not occur until the instar which reaches a critical head capsule width. In *T. ni*, the critical width is 1.66 mm (Jones, Jones, and Hammock, J. Insect Physiol., in press). The average head capsule width of parasitized 6th instars which molted to 7th instars was 1.73 ± 0.18 mm, which is above the critical width. Therefore, many of the supernumerary molts cannot be explained on

the basis of a subthreshold head capsule width in stunted, parasitized larvae.

In *M. sexta*, it has been shown that starvation of the last instar for several days, either after initial feeding or before feeding, will result in a supernumerary molt (Nijhout and Williams 1974, Nijhout 1975, Jones et al. 1981), and that this response is correlated with a starvation induced drastic decline in the trehalose titer (Jones et al., 1981). *M. sexta* parasitized by *Apanteles congregatus* often molt to a supernumerary instar (Beckage and Riddiford 1978), and *M. sexta* parasitized by *A. congregatus* also have below normal trehalose titers (Dahlman 1975). Such a situation generates the hypothesis that the supernumerary molts in parasitized *T. ni* were due to a drop in the hemolymph trehalose titer effected by parasite-induced nutrient stress. Unlike *M. sexta*, last-instar *T. ni* were found not to undergo a supernumerary molt when starved for several days either after initial feeding or before feeding, suggesting that even if the trehalose titer in parasite stressed *T. ni* drops below normal it will not cause a supernumerary molt in the host. Elimination of the head capsule width and trehalose models for supernumerary molting strengthens the hypothesis that the parasites are directly interfering with host endocrinology.

The mechanism by which parasitism by *C. truncatellum* causes the extra larval molt in the host is unknown. However, the *T. ni*-*C. truncatellum* interaction may provide a promising system for elucidating mechanisms of developmental regulation, specifically the intricacies of host-parasite interaction.

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