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Developmental and behavioural responses of larval *Trichoplusia ni* to parasitization by an imported braconid parasite *Chelonus* sp.

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ABSTRACT. Larval *Trichoplusia ni* (Hübner) (Noctuidae) parasitized by *Chelonus* sp. (near *curvimaculatus*) (Braconidae) precociously initiated pupation during the penultimate fourth instar. The temporal sequence of developmental markers exhibited by parasitized *T.ni* closely matched the temporal sequence in normal, pupating larvae. The parasitized larvae did not complete pupation, but consistently stopped development at a stage recognizable by a certain set of markers. This halt was observed in hosts from which parasites emerged and from hosts which had been stung but from which no parasites emerged. Weight gain and food consumption by parasitized hosts were significantly lower than normal, although most reached the fourth instar at the same time as normal larvae. Measurement of head capsule widths indicated that the width in precociously pupating larvae was less than the critical width associated with attainment of the pupation instar of normal larvae.

Introduction

The investigation of parasite disruption of host behaviour and metamorphosis is an increasingly popular field of study. The mode of disruption may take one of several forms including: (1) lengthening of duration of an instar, (2) induction of supernumerary instars, and (3) induction of precocious development by premature pupation, acquisition of adult structures, or diapause termination (Vinson & Iwantsch, 1980).

Parasitization may result in effects which impinge on the biological control and ecology of the host aside from its eventual death. If, for example, the parasitized hosts have increased feeding resulting from an extended larval stage or a supernumerary moult, then the utility of that parasite for biological control of the host may be diminished (Hunter & Stoner, 1975). On the other hand, if the parasitized hosts undergo premature cessation of feeding or precocious pupation, then the importance of the parasites in biological control of the host is enhanced (Vinson & Iwantsch, 1980). Also, changes in behaviour of parasitized hosts could put them in a different ecological niche from unparasitized hosts (Führer, 1968), and thus expose them to different mortality agents.

The developmental events leading to pupation and the endocrine bases for these events have been best studied in Lepidoptera. The occurrence of lengthened host instar duration or host supernumerary moults have been documented in parasitized Lepidoptera (Beckage & Riddiford, 1978; Fisher, 1971; Jones & Lewis, 1971; Jones *et al.*, 1981b; Miles & King, 1975; Rahman, 1970; Smilowitz & Iwantsch, 1973; Syme & Green, 1972). There have been numerous studies which reported, in passing, that there appeared to be precocious pupation in certain lepidopteran host–parasite systems (Broodryk, 1969; Butler, 1966; Luginbill, 1928; Pierce & Holloway, 1912; Rechav & Orion, 1975). Most of these reports have involved parasites of the genus *Chelonus*, but evidence for precocious pupation was limited to the observation that

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the parasitized host apparently spun a cocoon or dug a cell prior to parasite emergence. Alternatively, it is possible that such observed silk production or cell digging is a single parasite-induced event, and the other events occurring in normal pupation do not occur. In such a case, the parasite cannot be said to have truly induced precocious pupation in the host.

Since careful study to document the occurrence of precocious lepidopteran host pupation is lacking, the present investigation was performed to determine whether apparent cocoon spinning in penultimate instar larvae of *Trichoplusia ni* (Hübner) (Noctuidae) parasitized by *Chelonus* sp. is a single event or part of a normal physiological and behavioural sequence of pupation events which are precociously appearing in the host.

Materials and Methods

T.ni larvae were reared at 28°C in a 14 h light: 14 h dark regime as described by Shorey & Hale (1965) and Sparks *et al.* (1979). The parasite, *Chelonus* sp. near *curvimaculatus* (Braconidae), was derived from a stock collected from Ethiopia and generously provided by E. F. Legner. This species normally parasitized *Pectinophora gossypiella* (Saunders) (Gelechiidae) in Ethiopia. A group of parasitized *T.ni* eggs exposed to that parasite culture was used to start the colony involved in the present study. Eggs of *T.ni* oviposited on paper towelling were exposed to the parasites for several hours. The towelling was then removed and sections stapled to the underside of the lid of a 177-ml paper cup containing *T.ni* diet. The parasitized larvae were then reared under the same conditions as normal larvae.

For measurements involving daily weight gain, head capsule width, and duration of instars, larvae were selected from mass culture and isolated in 30-ml paper cups with diet. Weights and instar classification were recorded daily between 14 and 17 h after lights on, head capsules being measured across the widest point with an eyepiece micrometer.

For tests involving food consumption, larvae were selected from mass culture during their second instar while undergoing head capsule slippage to the third instar and placed individually in 30-ml plastic diet cups. These larvae were then removed from their cups at head capsule slippage to each subsequent instar. The cups of diet (with faeces removed) and a group of control diet cups containing no larvae were weighed on a Mettler balance accurate to 0.1 mg.

For tests involving developmental markers, normal and parasitized larvae were selected 14 h after lights-on, at such a weight that wandering would probably occur the next day. These larvae were isolated in 30-ml diet cups and, beginning the next day at 5 h after lights-on, were observed every 3–5 h for behavioural and morphological markers known to indicate sequential stages occurring prior to pupation (Jones *et al.*, 1981a).

Differences between means were separated using Duncan's new multiple range test with Kramer's modification for unequal sample sizes. Comparison of the percentage of larvae reaching various instars or developmental markers was done by applying a χ^2 test for homogeneity to each pair of groups for a given day post-egg hatch or for a given developmental marker.

Results

The weight gain of normal and parasitized larvae is given in Table 1. Parasites emerged from most (92%) but not all (8%) parasitized larvae. For this study parasitized larvae are those larvae from which a parasite emerged, while unsuccessfully parasitized larvae are those larvae which were apparently stung but from which no parasite emerged. These two groups were analysed separately. The weights of both groups were statistically indistinguishable from the control larvae at the time of head capsule slippage (HCS) to the third instar, but 2 days later, the normal larvae and unsuccessfully parasitized larvae weighed significantly more than parasitized larvae. This trend continued, but by day 4 post-second instar HCS, the normal larvae weighed significantly more than either parasitized group.

Normal larvae in mass culture together with parasitized larvae developed at the same rate as isolated normal control larvae, so data for normal larvae were taken from larvae selected

TABLE 1. Mean (± SD) weight (mg) of normal and parasitized *Trichoplusia ni* larvae at various intervals past second instar head capsule slippage.

Days past second instar head capsule slippage							
0	1	2	3	4	5	6	7
Normal larvae							
2.0 ± 0.3a	5.3 ± 1.4a	8.5 ± 2.1a	17.4 ± 4.6a	45.6 ± 13.3a	105.6 ± 39.9a	187.7 ± 53.6a	255.0 ± 25.8a
(17)	(9)	(17)	(17)	(17)	(11)	(7)	(5)
Parastitized larvae from which parasites emerged							
2.0 ± 0.3a	4.2 ± 1.2a	6.8 ± 1.5b	9.4 ± 2.4b	14.1 ± 4.4b	16.2 ± 4.2b	16.8 ± 3.7b	–
(17)	(29)	(37)	(37)	(37)	(37)	(19)	–
Stung but unsuccessfully parasitized larvae							
2.1 ± 0.5a	–	9.1 ± 1.7 a	17.1 ± 2.2a	26.8 ± 6.2c	40.7 ± 6.8c	52.1 ± 6.0c	55.6 ± 19.0b
(4)	–	(4)	(4)	(4)	(4)	(3)	(4)

Within each column, means followed by different letters are significantly different ($\alpha < 0.05$; Duncan's test). Figures in parentheses, number of larvae measured.

from the partly parasitized mass culture. The different rate of weight gain among the three groups provided the initial means of determining to which of the three groups a larva belonged, but this status was always confirmed by post-experiment observation, and larvae initially classified wrongly transferred to their correct group.

The rates of development of normal and parasitized larvae were essentially the same for the first 7 days following egg hatch (i.e. until about early third instar; Table 2). After this time, some (25%) parasitized larvae began lagging a day behind normal, concomitant with the increasing divergence in weight gain between parasitized and normal larvae. By 11 days post-egg hatch, most normal larvae had reached the fifth instar, while most parasitized larvae had begun silk (apparent cocoon) spinning behaviour as fourth instars. The unsuccessfully parasitized larvae usually achieved a stunted fifth instar prior to spinning. Thus, most parasitized larvae, though small in size, reached the mid-fourth instar at the same time as did normal larvae. Instead of then undergoing apolysis to the fifth instar, however, they apparently began spinning a cocoon.

To monitor the behavioural and physiological events in such larvae more closely, mid-fourth instar parasitized and mid-fifth instar normal larvae were selected as near wandering (searching for pupation site), on the basis of weight, and their development observed. The data in Fig. 1 show that both normal and parasitized larvae began to exhibit behavioural and developmental markers associated with pupation (Jones *et al.*, 1981a) at the same time of day. Normal larvae turned from white-grey to a uniform green colour after cessation of feeding and migrated to the top of the container, Parasitized larvae, instead of turning green, turned a uniform white colour, and also migrated upward. By 5 h after lights-on, a similar percentage of both groups had migrated upward and produced a white faecal pellet. By 13 h after lights-on, the majority of both groups had either begun to spin or had spun a dense cocoon-like enclosure. By 17 h after lights-on, most larvae in both groups had spun the cocoon.

After 17 h the rate of development of parasitized larvae was much slower than normal. The normal larvae proceeded to go rapidly through ocellar pigment withdrawal, crochet retraction, loss of ability to walk or curl, ocellar white spot migration, and finally moulting. The parasitized larvae, on the other hand, retained the ability to curl, never more than partially retracted their crochets, and did not show more than slight ocellar pigment withdrawal until just before parasite emergence.

TABLE 2. The percentage distribution (post hatch) of normal and parasitized *T.ni* larvae as they pass through successive instars.

Instar	Days post-egg hatch													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Parasitized larvae (n = 19)														
1	100	100	88	24	–	–	–	–	–	–	–	–	–	–
2	–	–	12	76	92	29	7	4	–	–	–	–	–	–
3	–	–	–	–	8	71	89	49	12	7	–	–	–	–
4	–	–	–	–	–	–	4	47	84	60	19	10	2	–
4 Spin.	–	–	–	–	–	–	–	–	4	33	60	46	36	–
Par. Em.	–	–	–	–	–	–	–	–	–	–	7	31	52	65
5	–	–	–	–	–	–	–	–	–	–	12	11	4	13
5 Spin.	–	–	–	–	–	–	–	–	–	–	–	–	5	18
Par. Em.	–	–	–	–	–	–	–	–	–	–	–	–	–	4
Normal, unparasitized larvae (n = 18)														
1	100	100	67	11	–	–	–	–	–	–	–	–		
2	–	–	33	89	89	17	–	–	–	–	–	–		
3	–	–	–	–	11	83	94	28	6	7	–	–		
4	–	–	–	–	–	–	6	72	94	73	22	11		
5	–	–	–	–	–	–	–	–	–	20	78	89		

4 Spin. and 5 Spin. = host spinning cocoon; Par. Em. = parasite emerged from host.

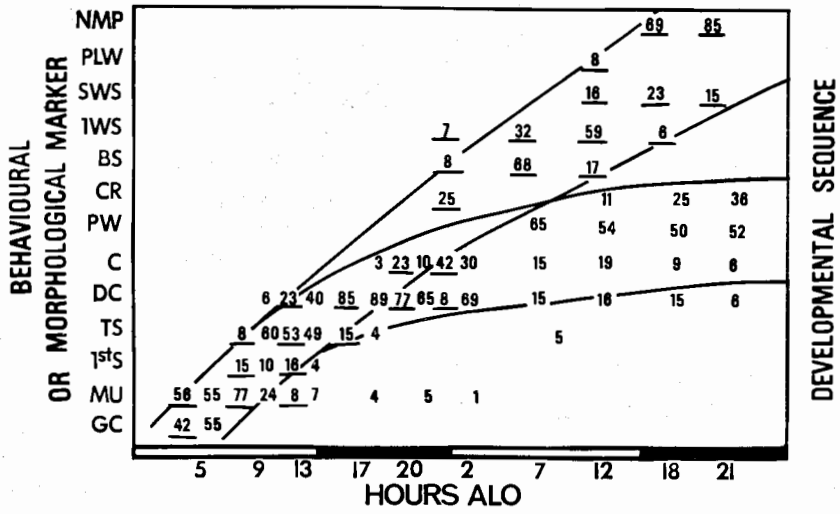

FIG. 1. Percentage of larvae attaining various developmental markers at different times from cessation of feeding through to the moult to a pupa. Underlined percentages are for normal larvae (n = 14); percentages not underlined are for both successfully and unsuccessfully parasitized larvae (n = 40) since there was no statistical difference between the two groups. ALO = after lights on; GC = green (normal larvae) or uniform white (parasitized larvae) colour; MU = migration upward; 1stS = first silk spinning; TS = thin silk cocoon; DC = dense cocoon; C = larvae can curl but not walk; PW = pigment withdrawal from ocelli; CR = crochet retraction; BS = large black eye spot has migrated to hind margin of head capsule; 1WS = one white ocellar spot has migrated to hind margin of head capsule; SWS = several white spots at hind margin of head capsule; PLW = prolegs wither; NMP = newly moulted pupa.

Many of the parasitized larvae classified as unable to walk may have been hindered from doing so by the presence within them of a large parasite, rather than because of a developmental signal. Those larvae that could walk did so by more of a squirming walk than a looping walk, and appeared unable to bend their posterior half (containing the parasite) to make a looping movement. Some larvae apparently underwent partial crochet retraction prior to, instead of after, partial ocellar pigment retraction, but this sequence of events may have been due to the difficulty in obtaining a well-defined line between partial occurrence of these events and none. Dissection revealed that precociously pupating fourth instars had much larger than normal fat bodies, and that these organs more resembled those in a pupating normal fifth instar larva than those in a normal fourth instar larva.

By the fourth instar head capsule widths of parasitized larvae from which parasites emerged were significantly less than for normal fourth instars (Table 3). Host larvae from which parasites did not emerge had a normal head capsule width through the fourth instar, but by the fifth instar (if they developed that far) their head capsule was significantly smaller than normal.

The food consumption of parasitized larvae from which parasites emerged was indistinguishable from normal during the third instar. However, parasitized fourth instars ate significantly less, and since these hosts did not survive into the fifth instar, their total food consumption was significantly less than normal (Table 4).

Only three of fifteen *Chelonus* larvae separated from their host shortly after they emerged from it survived to pupate. However, all of another fifteen *Chelonus* allowed to feed externally on their host after they had emerged from it did pupate, indicating that such feeding is important for this parasite's survival.

Discussion

The present study provides strong evidence that parasitized fourth instar *T.ni* larvae actually precociously initiate apparently normal metamorphic development. The change

TABLE 3. Mean (±SD) head capsule width (mm) in various instars of normal and *Chelonus*-parasitized *T.ni* (conventions as in Table 1).

Instar			
2	3	4	5
Normal larvae			
0.47 ± 0.03a	0.69 ± 0.06a	1.07 ± 0.09a	1.62 ± 0.22a
(11)	(13)	(11)	(9)
Parasitized larvae			
0.45 ± 0.03a	0.68 ± 0.03a	0.96 ± 0.06b	–
(14)	(17)	(21)	–
Stung but unsuccessfully parasitized larvae			
0.50 ± 0.06a	0.65 ± 0.05a	1.06 ± 0.04a	1.34 ± 0.07b
(4)	(4)	(4)	(20)

TABLE 4. Mean (±SD) food consumption (g) in various instars of normal and *Chelonus*-parasitized *T.ni* (conventions as in Table 1).

Instar		
3	4	5
Normal larvae		
0.07 ± 0.04a	0.25 ± 0.10a	0.79 ± 0.11a
(11)	(11)	(10)
Parasitized larvae		
0.07 ± 0.03a	0.08 ± 0.05b	0*
(27)	(32)	–

*Larvae died in fourth instar.

in colour, migration upward, production of a white faecal pellet and cocoon spinning is a sequence observed only in pupating larvae. Moreover, the temporal sequence of these events in parasitized larvae closely matched the sequence occurring in normal larvae pupating in the fifth instar. Further, the abnormally and extensively developed fat body in parasitized fourth instar larvae closely resembled that occurring in normal, pupating larvae. Thus, the apparent precocious cocoon spinning or pupation cell digging by hosts, also observed in other *Chelonus*–Lepidoptera host interactions, is probably a true precocious pupation phenomenon.

An intriguing question is posed by those unsuccessfully parasitized larvae which precociously began the pupation sequence but from which no parasites ever emerged. One possibility is that no egg was oviposited in these hosts by the female parasite or that it was quickly destroyed by the host and the 'parasitization' effect was caused by injection of fluid from one of the several glands associated with oviposition (Vinson & Iwantsch, 1980). Previous work on delayed effects of parasite venom indicate a delay of at most 6–8 days, and this apparently within a single instar (DeLeon, 1935; Genieys, 1925; Munro, 1917). Thus, the very delayed nature of the precocious *T.ni* pupation (i.e. the egg stage and four instars or 11–13 days elapsed prior to the effect), would seem to argue against the effect being simply a result of fluid injected into the host egg by the female. Another possible explanation is that the effect resulted from the presence of a parasite larva, which in some hosts was overcome after the sequence of events leading to precocious host metamorphosis had been initiated.

Several aspects of this study impinge upon the usefulness of this kind of parasite in biological control of hosts such as *T.ni*, which is a pest of vegetables and other crops. Behavioural changes in *T.ni* due to *Chelonus*-parasitization significantly decrease its food consumption and, thus, its potential damage to crops. Also, the nature of the interference with *T.ni* development is such that even hosts which have apparently rejected the parasite will still precociously cease feeding, in order to initiate pupation. Messenger & van den Bosch (1971) indicate that parasites are of no use in the control of an immune host species. This may be true when the host is immune from both the parasite and its effects on the host. However, when the nature of the host–parasite interaction is such that the parasitized immune individuals still suffer the endocrine disturbances of successful parasitization, the

parasite may have a greater effect on the host density than might be suspected from the percentage of the host population which dies due to parasite emergence.

The manner in which parasitism results in precocious initiation of pupation in the host is not known. A pervasive theme in the literature is to conclude that physiological disturbances in parasitized hosts are examples of host regulation by the parasite, especially when the disturbance is, teleologically speaking, to the parasite's advantage. However, 'regulation' implies an active process on the part of the parasite in interfering with the host's physiology, and the presentation of only teleological arguments in support of such hypotheses is not really valid. Thus, regulation should be distinguished from passive, indirect, or 'unintentional' disturbances of the host's physiology.

By conventional analysis of the interaction examined in the present study, as well as in other *Chelonus*–host interactions examined previously, it might be hypothesized to be the parasite's advantage to cause precocious cocoon-spinning in the host and yet not allow the sequence to proceed to host pupation. Host cocoon spinning results in the parasite emerging and pupating in a protected environment. If the host were to continue the pupation sequence, the internal parasite might find itself in a milieu not suitable for further development. In fact, some braconid larval parasites of noctuids do not survive when placed in a prepupal or pupal host (Lewis, 1970).

Before the nature of the disturbance of normal metamorphosis in larvae parasitized by *Chelonus* can be described as regulation of the host, however, the simpler explanation of indirect disturbance must first be disproved. Data from the present study allow the rejection of several such models. It has been shown for Lepidoptera that the pupation instar is not reached until the head capsule achieves a critical size (Decker & Maddox, 1971; Nijhout, 1975). The threshold size in *T.ni* is a width of 1.66 mm (Jones *et al.*, 1981a). The head capsule width of cocoon-spinning parasitized fourth or fifth instar larvae was well below this threshold (Table 3). Thus, the mechanism by which parasitism induced precocious initiation of metamorphosis is not by causing an abnormally large penultimate instar head capsule size. A second indirect mechanism could be the indiscriminate consumption of the host corpora allata (along with other tissue) by the parasite, resulting in a drop in the host's juvenile hormone titre. The absence of juvenile hormone during the next release of ecdysone would result in the host becoming precociously programmed to begin metamorphosis. However, dissection of parasitized cocoon-spinning larvae revealed intact host corpora allata.

Elimination of these models for indirect disturbance of host physiology strengthens the possibility that direct host regulation is involved. The mechanism by which the parasite may be initiating precocious pupation in the host and also blocking its completion is as yet unknown. However, the *Chelonus* sp.–*Trichoplusia ni* system offers a new and intriguing arena for investigation of endocrine regulation of development and of host–parasite interaction.

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