INFLUENCE OF ENDOCRINE ACTIVITY ON LARVAL DEVELOPMENT IN *BUSSEOLA FUSCA* (FULLER)(LEPIDOPTERA:NOCTUIDAE)

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Abstract

The present study investigated the role of juvenile and moulting hormones in development of Busseola fusca. *Morphometric measurements were used to distinguish differences in endocrine activity between non-diapause and diapause development with regard to the prothoracic glands and the* corpora allata. *The* corpora allata *of non-diapause larvae were similar in size to those of diapause larvae. The critical period for the prothoracic glands to produce the required titer of ecdysone for further development was by the third day after ecdysis to the last larval instar. Juvenile hormone extracts from haemolymph of* Busseola fusca *larvae in non-diapause and diapause development had morphometric effects on last instar nymphs of* Dysdercus cingulatus. *The extent of juvenilization of nymphs by these extracts was a measure of relative titer of juvenile hormone present. Extracts of JH from the fifth instar larvae of* Busseola fusc*a and larvae in early diapause gave higher juvenilizing scores than JH from non-diapause sixth instar larvae.*

INTRODUCTION

Larval development in the Lepidoptera is regulated by many factors, some of which are endocrine in nature. These include the horrnones, which regulate the processes of molting and metamorphosis. Larval molt occurs in the presence of a molting hormone (ecdysone) and juvenile hormone (JH). During larval development, ecdysone is required for all types of molting but JH secreted by the corpora allata (CA) is present only when the genetic programming of the insect requires growth without differentiation. Thus JH inhibits metamorphosis during immature stages. At metamorphosis, the larva molts into a pupa and finally to adult. Metamorphic molts take place in negligible titers of JH.

The present study investigated the endocrine system in non-diapause and diapause larvae with respect to morphometric characteristics of the CA and

prothoracic glands (PG), estimation of JH titers and the critical period for the glands to exert their influence on pupation.

MATERIALS AND METHODS

Morphometric studies on the CA and PG

Morphometric studies of the CA and PG were undertaken on non-diapause larvae of *Busseola fusca* in different states. These states were early- (immediately after molting), mid- (on the fourth day) and late- (on the seventh day) of the last instar non-diapause larvae, and diapausing larvae in early(July), mid- (September) and late- (November) diapause. Larvae were anaesthetized in diethyl ether and dissected under insect Ringer saline (Ephrussi & Beadle 1936). The maximum width of the left and right prothoracic glands was measured using an ocular micrometer.

Determination of haemolymph JH titer

The relative titers of JH in the haemolymph of the penultimate (5th) instar and in the last nondiapause and diapause larval instars were determined indirectly by assaying hexane extracts of the haemolyraph for JH activity on the last instar nymphs of *Dysdercus cingulatus.*

Critical period for the release of ecdysone

Abdomens were isolated from the anterior parts of the body which contain the endocrine glands. This was done by ligation applied between the thorax and the abdomen. Ligation was applied at daily intervals in the last larval molt. Further, development of the isolated abdomen was studied. The number of larvae ligated on each day after the last larval molt was 21. The ligated larvae were maintained individually in plastic jam cups with perforated lids and containing a piece of moist Kleenex tissue paper. Observations were made on the abdomens until they pupated or for a period of three weeks. The number and percentage of abdomens pupating in each group was recorded.

Extraction and bioassay of JH

Haemolymph samples were collected from the 5th instar, early and late (prepupa), mixed with hexane and vortexed vigorously and centriftiged at 4000 rpm for 15 minutes, at 4°C. The hexane epiphase was pipetted out and the solvent removed by evaporation over a water bath. The extraction procedure of each sample was repeated twice. The resultant residue was stored at 4°C until the time of the bioassay.

The hexane extract of the haemolymph was diluted 10, 100 and 1,000 times (volume/volume) in acetone. These dilutions were assayed for morphogenetic effects on nymphs of *D. cingulatus.* Freshly molted fifth instar nymphs from a laboratory colony were anaesthetized in diethyl-ether, and 1 µl of the test solution was applied topically. There were three replicates of five insects each for every dose. For control, nymphs were similarly treated with l µl acetone.

The treated and control insects were fed on cotton seeds soaked in water until they died. They were also examined for morphogenetic effects. The morphogenetic effects were measured quantitatively using a score of 0-3, based on the degree of the development of the fore wings and the number of tarsal segments. The scores were defined as follow:

(i) score 0: adults, those with three tarsal segments and fully differentiated wings with horizontal bars.
(ii) score 1 -2: Nymphs showing varying degrees of larval and adult characters (intermediate)
(iii) score 3: larval characteristics , supernumerary with two tarsal segments as in nymphs, short and less developed fore wings without the horizontal bars

RESULTS AND DISCUSSION

Morphometric sizes of the CA and PG

There were no significant differences in sizes of the CA of non-diapause and diapause larvae or of the CA of males and females (Table 1). In non-diapause larvae, the width of the PG was significantly smaller ($P < 0.05$) than that of diapause larvae. There were no significant differences in the widths of the PG for both males and females in either condition($P > 0.05$).

Table 1. Mean diameters of CA and PG in non-diapause and diapause larvae of *Busseola fusca*

Developmental state	Sex[1]	Mean diameter = Å(μM units)	
		CA	PG
Non-diapause	male[1]	0.336	0.347[a,2]
	female[1]	0.339	0.376[a]
Diapause	male[1]	0.456	0.330[b]
	female[1]	0.469	0.284 [b]

[1]no. larvae used = 8

[2]means in same column followed by same alphabet are not significantly different from each other

Critical period for the release of ecdysone

The effect of post ecdysial age at abdominal ligation of the last instar larvae on subsequent development of the isolated abdomens is given in Table 2. None of the abdomens isolated from the head and thorax within two days after molting pupated. Less than a quarter of abdomens isolated 3 days after molting pupated. As the larvae aged, there was a gradual increase in the % of abdomens developing in the absence of the head and the thorax. Thus 23-98% of the abdomens ligated off 3-7 days after the previous molt pupated. More than 50% of the abdomens pupated without PG after a period of 5 days.

Table 2. The effects of age at ligation on pupation of isolated (ligated) abdomens of the last instar larvae of *Busseola fusca*

Larval age at ligation (days)	% abdomens pupated (means ± s.e.)
0	0.0
1	0.0
2	23.3 ± 1.17
3	27.0 ± 2.24
4	60.0 ± 2.58
5	90.0 ± 2.24
6	98.0 ± 0.75

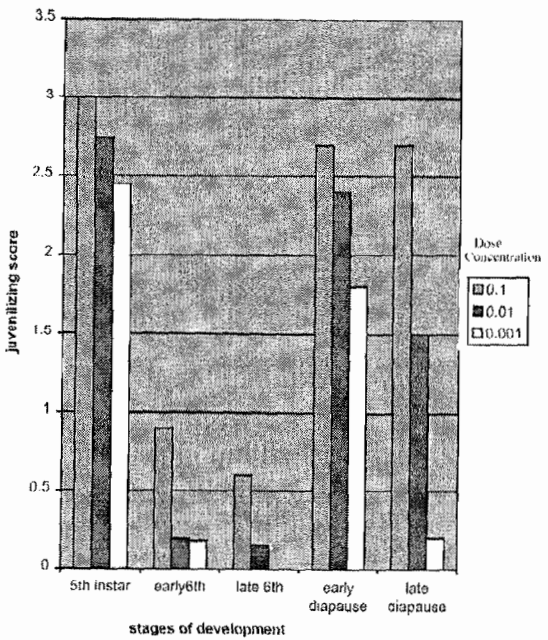

Fig. 1: Juvenilizing effects of haemolymph extracts of *Busseola fusca* on the development of the last instar nymphs of *Dysdercus cingulatus*

Morphogenetic effects of haemolymph JH extracts of penultimate and last instar nondiapause and diapause larvae of *B. fusca* are given in Figure 1. Super numerary larvae molt (average score, 3) was induced in the 5th instar of *D. cingulatus* treated with 1 µl of 10 times diluted haemolymph extract of the penultimate (5th instar) larvae of *B. fusca*. Nymphs which were treated with an extract diluted 1,000 times gave an average score of 2.8. From these results it can be deduced that the titer of JH in the haemolymph of the fifth instar larvae of *B. fusca* was high. In contrast, the average juvenilizing effect of the corresponding score of the extract from the sixth instar, non-diapause larvae was only 0.8. Most of the treated nymphs molted into slightly malformed adults. These results showed that the extracts from the non-diapausing sixth instar larvae of *B. fusca* had a very low titer of JH. Extracts from early diapausing larvae diluted 10, 100 and 1,000 times showed average juvenilizing scores of 2.8, 2.4 and 1. 8, respectively. Similarly, the corresponding dilutions of extracts ftom late diapausing larvae resulted to average scores of 2.8, 1.2 and 0.2. These results indicate that diapause larvae contain a higher titer of JH than the non-diapause last instar larvae. Also, the titer of JH in late diapause larvae was comparatively lower than during early diapause.

The size of the CA has been used before as one of the criteria to assess their activity patterns (Odhiambo 1966, Lanzrein *et al.* 1978). In the present study, the absence of significant differences in size of the CA of the non-diapause and diapause larvae shows that morphological studies alone may be inadequate to measure the secretory activity of these glands in larvae of *B. fusca.*

The CA of diapause last instar larvae of *B. fusca* normally retain secretory activity. Therefore, the slightly larger size of the CA of the diapausing larvae relative to that of the nondiapausing larvae is a sign of increasing secretory activity. Correlation between the size of the cells of the prothoracic glands and their activity has been reported in many insects. For example, bigger cells were reported during maximum ecdysone synthesis in the army worm *Spodoptera litoralis* (Zimowoska *et al.* 1985). In *B. fusca,* the prothoracic glands are probably less active during the diapause state but they are not refractory to stimulatory agents in the haemolymph. In fact occasionally, the glands become active and produce enough moulting hormone to effect stationary moults.

The ligation procedure is useful for investigating the role of critical period of activity of the neuroendocrine system (Kiduchi & Raddiford 1978). By ligation, the brain, corpora allata and prothoracic glands were separated from the rest of the body of the larvae, thus leaving the abdomens devoid of further source of the hormones from these glands. Depending on the time of ligation, an abdomen of the last instar larva can either remain as it was or continue to develop. Further development of the abdomen depends on the presence of the molting hormone in the haemolymph. Under normal conditions, ecdysone production by the glands depends on their stimulation from the brain. This is

facilitated by the Prothoracicotropic (PTTH) produced in the brain. Thus, the brain is required, at least for some critical period during, the last instar. The present experiment did not reveal the critical period for the stimulation by the brain through PTTH, but it appeared to be within the first three days of the molt because, then, sufficient quantity of ecdysone appeared to be present in the haemolymph to induce pupation in more than 20% of the isolated abdomens.

The low levels in the titer of juvenile hormone in the haemolymph are typical of the last instar larvae of most lepidopteran species (Bollenbacher 1988). Low levels of juvenile hormone in the haemolymph affect activities of other endocrine glands. For example, declining titers of JH in *Manduca sexta* is necessary for a small release of PTTH that triggers synthesis and release of ecdysone by prothoracic glands (Riddiford & Truman 1978).

Unlike in last instar larvae of other lepidoptera (Riddiford 1985), the JH titer in the final instar larvae of *B. fusca* remained low throughout the stadium. The declining JH titers has also been reported in other species with life similar history patterns as *B. fusca*. This includes, the South western corn borer, *Diatraea grandiosella* (Yin & Chippendale 1976) and the European corn borer, *Ostrinia nubilalis* (Beck & Shane 1973). However, in some species, JH titer rises during the post commitment phase to prevent precocious development of adult characters during pupation (Kiguchi & Riddiford 1978, Whisenton *et al.* 1987).

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