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Juvenile Hormone Esterases of Lepidoptera

I. Activity in the Hemolymph During the Last Larval Instar of 11 Species

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Summary. The juvenile hormone esterase (JHE) titer was measured during the last larval instar of 11 species of Lepidoptera (*Pieris rapae, Junonia coenia, Danaus plexippus, Hemileuca nevadensis, Pectinophora gossypiella, Spodoptera exigua, Orgyia vetusta, Ephestia elutella, Galleria mellonella, Manduca sexta* and *Estigmene acrea*). All species had a peak of JHE at or near the time of wandering. The peak activity at this time ranged from 0.8 to 388 nmoles JH III cleaved/min·ml. All species except *J. coenia* had a second peak of JHE during the late prepupal stage. The height of the second peak ranged from 0.4 to 98.4 nmoles/min·ml. However, there was no apparent correlation between size of the first and second JHE activity peaks for the lepidopteran species examined. There was an apparent relationship between the height of the first and second JHE peaks and reports on titer of JH just prior to these peaks. These data support, with some qualifications, the extension of developmental information obtained on several well studied species to a variety of Lepidoptera.

Introduction

In holometabolous insects high titers of the juvenile hormones (JH's) are thought responsible for maintaining the larval stages. Low JH titers cause a shift from isometric to anisometric development leading to the pupal and adult stages. Degradation of JH by ester hydrolysis is the major initial pathway of JH metabolism in the Lepidoptera examined and has been suggested as a mode of JH titer regulation necessary for normal development (Gilbert et al. 1978). In fact, recent data are consistent with a model in which JH biosynthesis by the corpora allata is reduced but not halted, and the low JH titers necessary for prothoracicotropic hormone effects result from increased JH hydrolysis (Sparks and Hammock 1980). The first lepidopteran titered during the last larval instar for hemolymph JH esterase (JHE) activity (*Manduca sexta* L.) possessed two peaks of JHE, each correlated with an abrupt decline in hemolymph JH (Weirich et al. 1973; Nijhout and Williams 1974; Vince and Gilbert 1977; K. Judy, cited by Sridhara et al. 1978), although Nijhout (1975) noted an initial JH decline in last instar *Manduca sexta* may not be due to increased JHE activity. Other lepidopteran species were subsequently found to possess a last larval instar JH profile similar to *Manduca sexta* (initially high, falling very low and a prepupal rise; Varjas et al. 1976; Yagi 1976; Hsiao and Hsiao 1977; Mauchamp et al. 1979). The correlation of JHE activity with the JH declines in *Manduca sexta* prompted many researchers to generate hypotheses about JH titer regulation in Lepidoptera in general, based on the *Manduca sexta* model (Riddiford 1980). The applicability of this model to Lepidoptera was supported by the report of two similarly placed peaks of JHE activity in last larval instar *Trichoplusia ni* (Hübner) (Sparks et al. 1979) and by the demonstration of the importance of these peaks for normal development to occur (Sparks and Hammock 1980). However, *Galleria mellonella* (L.) was found to lack the prepupal peak of JHE activity (Hwang-Hsu et al. 1979), although it possessed the prepupal burst of JH (Hsiao and Hsiao 1977). The *Galleria mellonella* JHE profile calls into question the applicability of the *Manduca sexta* model to all Lepidoptera.

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Abbreviations: JH juvenile hormone; *JHE* juvenile hormone esterase; *PTTH* prothoracotropic hormone; *Ro-10-3108* 1-(4′-ethylphenoxy)-6,7-epoxy-3-ethyl-7-methylnonane

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The relative heights of the two last instar peaks of JHE activity in *Manduca sexta* also may not be an appropriate model for all Lepidoptera. In *Manduca sexta* the first peak in JHE activity is much greater than the second peak, while in *Trichoplusia ni* the heights of these two peaks are about the same. If the amount of JHE activity is an indicator of the importance of JH ester hydrolysis in regulation of the JH titer, then JHE may be more important for eliminating prepupal JH in *Trichoplusia ni* than in *Manduca sexta*.

It also appears that the first peak of JHE activity in the final instar of *Manduca sexta, Trichoplusia ni* and *Galleria mellonella* is under neurosecretory control (Vince and Gilbert 1977; McCaleb and Kumaran 1979; G. Jones et al. 1981; Kumaran et al. 1981) while the second, prepupal peak of JHE in the first two species is induced by a burst of JH from the corpora allata (Hammock et al. 1981). If the pattern of JHE activity during the last instar of other Lepidoptera is similar to that of *Manduca sexta* and *Trichoplusia ni*, then the JHE regulatory mechanisms found in *Manduca sexta* and *Trichoplusia ni* may apply to the other species as well.

If the interaction between JH and JHE titers during the last instar in *Manduca sexta* is to serve as a comprehensive model for the Lepidoptera, with *Galleria mellonella* being an exceptional species, the JHE titer in many more lepidopteran species must be examined and compared to that in *Manduca sexta*. Thus a project to monitor hemolymph JHE activity during the last instar of 11 species of Lepidoptera representing 10 families was undertaken, and the results are reported here. The biochemical properties of the hemolymph binding proteins and JH esterases from some of these species are discussed in a companion manuscript (Wing et al., in preparation).

Materials and Methods

Species used in the study, their source and rearing conditions are given in Table 1. All weighings and bleedings were made between 14:00 and 17:00 h (day begins at lights on, 5:00 h a.m.), except where otherwise noted. Each larva was bled only once. Developmental markers were used whenever possible to stage and precisely time subject animals in an effort to insure that points of highest hemolymph JHE activity would not be missed. D. Jones et al. (1981) previously found that use of devel-

Table 1. Species, source of species, and rearing conditions of larvae titered for JH esterase during the last larval instar[a]

Family	Species	Source	Diet medium
Pieridae	*Pieris rapae* (cabbage butterfly)	F_1 progeny of field adults	*Brassica*
Nymphalidae[b]	*Junonia coenia* (buckeye butterfly)	Laboratory culture	*Passiflora* or *Althaea*
Danaidae	*Danaus plexippus* (monarch butterfly)	Field collected larvae	*Asclepias*
Saturniidae	*Hemileuca nevadensis* (Nevada buck-moth)	Field collected larvae	*Salix*
Gelechiidae	*Pectinophora gossypiella* (pink bollworm)	Laboratory culture	Wheat germ base
Noctuidae	*Spodoptera exigua* (beet armyworm)	Laboratory culture	Pinto bean base
Liparidae	*Orgyia vetusta* (western tussock moth)	Field collected larvae	*Erigonum*
Pyralidae	*Ephestia elutella* (tobacco moth)	Laboratory culture	Bran-wheat germ base
Pyralidae	*Galleria mellonella* (greater wax moth)	Laboratory culture	Cereal[c]
Arctiidae	*Estigmene acrea* (salt marsh caterpillar)	F_1 progeny of field larvae	Pinto bean base
Sphingidae[b]	*Manduca sexta* (tobacco hornworm)	Laboratory culture	Corn grit base

[a] All larvae reared under 14 light: 10 dark, 28 °C conditions, except for *G. mellonella* which were kept in total darkness
[b] Diet and larvae purchased from Carolina Biological Supplies
[c] Bhaskaran, personal communication

opmental markers was helpful in staging *Trichoplusia ni* larvae used in determining levels of last instar JHE activity. It is recognized that rearing conditions, photoperiod, temperature, etc. can affect the observed JHE activity. Thus, although hemolymph JHE activity in *Galleria mellonella* and *Manduca sexta* has been reported by other laboratories, these two species were monitored again here using animals reared in this laboratory and our assay procedure to facilitate comparisons.

JHE activity was monitored using ^{3}H-C10 labeled JH III (New England Nuclear, 11 Ci/mmole or 470 GBq/nmole) as a substrate as previously described (Hammock and Sparks 1977). Hemolymph was diluted with sodium phosphate buffer (pH = 7.4, I = 0.2 M + 0.01% phenylthiourea) and held on ice at 4 °C until assayed (usually within 48 h). Several authors have found that in *Manduca sexta* there is a broad pH optimum for JHE activity over several pH units (Weirich et al. 1973; Vince and Gilbert 1977), and this is also true for JHE activity in *Trichoplusia ni* (Wing et al. 1981), *Heliothis virescens, Spodoptera exigua* and *Galleria mellonella* (unp. data). Therefore, observed differences in JHE activities among species probably reflect true differences and are not artifacts of hypothetically different pH optima. The hemolymph was diluted with buffer such that the appearance of JH acid was linear with time during the course of the assay. In each case, the appearance of JH acid was dependent on time and hemolymph concentration. Assays were performed at 30 °C with 5×10^{-6} M substrate added in ethanol. Each larva served as a single replication for the time point at which it was bled. The number of replications was variable, but the points of highest and lowest activity received the most replications (see figure legends for more details).

Statistical multiple comparisons were made to determine the number of peaks of hemolymph JHE activity during the last larval instar, to compare the amounts of activity of the two peaks within a species (if there was more than one peak), and to compare peaks from different species. For the purposes of this study a peak is defined as a statistically significant rise in activity as compared with activities at adjacent (though not necessarily immediately adjacent) time points. However, the skewed nature of the data, the unequal variances and unequal sample sizes meant that the assumptions for parametric procedures were usually not satisfied. Therefore, the nonparametric test which is least sensitive to skewness in distributions and unequal variances of sampled populations (median test) was used to determine if there was a difference among the central locations (medians) of the sampled populations. The median test was provided with an experimentwise error rate similar to that of Duncan's new multiple range test, a common parametric procedure by use of the Bonferroni Inequality. The number of statistically significant JHE activity peaks declared for each species with the median test was the same as when checked with parametric procedures. Variation among replications in each time point is reported as standard deviation.

Results

Pieris rapae (L.). The duration of and weight gain during the last instar are given in Fig. 1a. After cessation of feeding, weight was not used to stage larvae but instead the following developmental markers were used: migration to top of rearing containers, back girdle formation (Smith and Smilowitz 1976), loss of ability to walk, loss of ability to curl, newly molted soft pupa, soft pupa with sclerotized horns, 24 h old pupa.

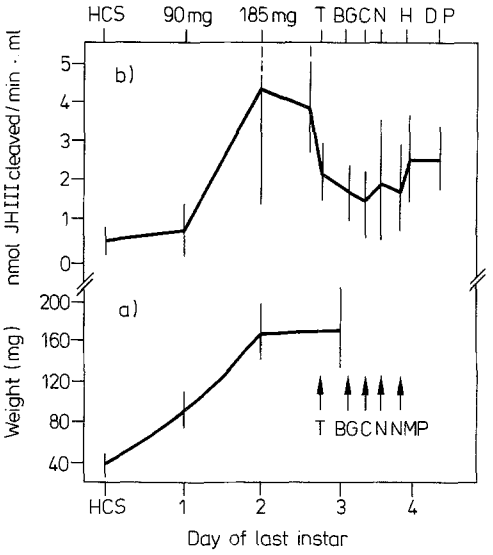

Fig. 1. a Weight gain and physiological markers observed during the last larval instar of *Pieris rapae* (*T* migration to top of container; *BG* back girdle formation; *C* can curl, not walk; *N* not able to curl; *NMP* newly molted pupa; *H* soft pupa, horns sclerotized; D_1P day 1 pupa). **b** Hemolymph JH esterase activity during the last instar ($n = 4$–14 replicates per time point)

The profile of JHE activity during the last instar and early pupa is given in Fig. 1b. Two statistically significant peaks were found, one occurring near the time of maximum larval weight, and the second peak, more difficult to detect, occurring close to pupal ecdysis. The first peak was significantly higher than the second peak.

Junonia coenia (Hübner). The duration of and weight gain during the last instar are given in Fig. 2a. There appeared to be a bimodal distribution in maximum weight achieved, perhaps due to sexual dimorphism, but since it was not severe it was not further investigated. After cessation of feeding, developmental markers were used to stage the larvae and these included: migration to top of rearing container, first hanging from container top, first hanging +6 h, first hanging +12 h, newly molted wet pupa, semi-sclerotized pupa (~12 h old), and day 1 pupa.

The profile of JHE activity during the last instar and early pupa is given in Fig. 2b. A slow rise to a single statistically significant peak was found which reached a maximum 6 h after first hanging from the top of the rearing container. The titer declined back to a low point (12 h pupa) and appeared to rise again through day 3 pupal stage. This titer profile was replicated on 4 different generations of larvae.

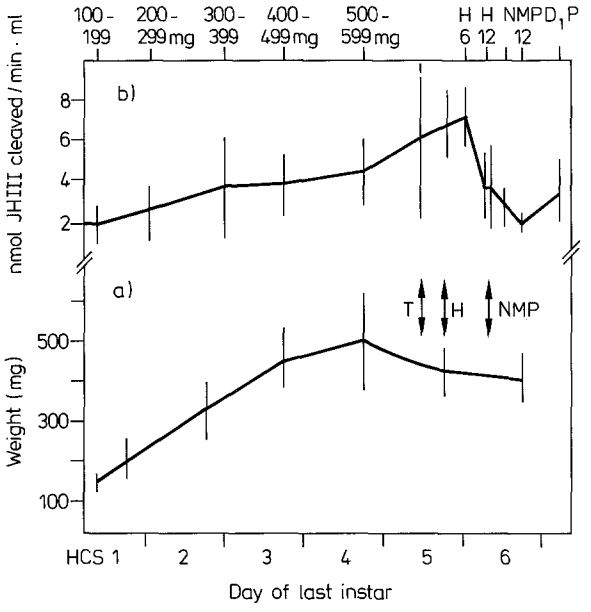

Fig. 2. a Weight gain and physiological markers observed during the last larval instar of *Junonia coenia* (*T* migration to top of container; *H* hanging by tip of abdomen; *NMP* newly molted pupa; D_1P day 1 pupa). **b** Hemolymph JH esterase activity during the last larval instar and early pupa (*H*+*6* hanging for 6 h, etc.) (n= 5 to 18)

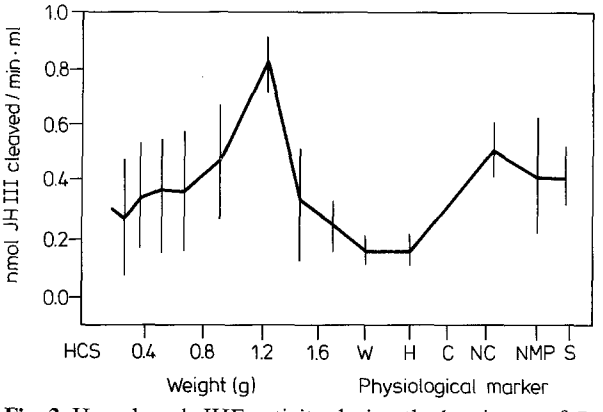

Fig. 3. Hemolymph JHE activity during the last instar of *Danaus plexippus* (*HCS* head capsule slippage; *W* wandering stage; *H* hanging from top of container; *C* able to curl, but not walk; *NC* not able to curl up; *NMP* newly molted pupa; *S* pigmented but still soft pupa) (n=3 to 9)

Danaus plexippus (L.). During the feeding stage, larvae were synchronized by weight while post-feeding larvae were synchronized by the following developmental markers: wandering, migration to top of rearing chamber, hanging by terminal prolegs but still able to walk, loss of ability to walk, loss of ability to curl, newly molted pupa, soft pupa with some pupal coloration.

The profile of JHE activity during the last instar is given in Fig. 3. Activity was very low, yet two statistically significant peaks were found. The first peak occurred definitely prior to achievement of maximum weight and the second during the late prepupal stage. The heights of the two peaks were not significantly different.

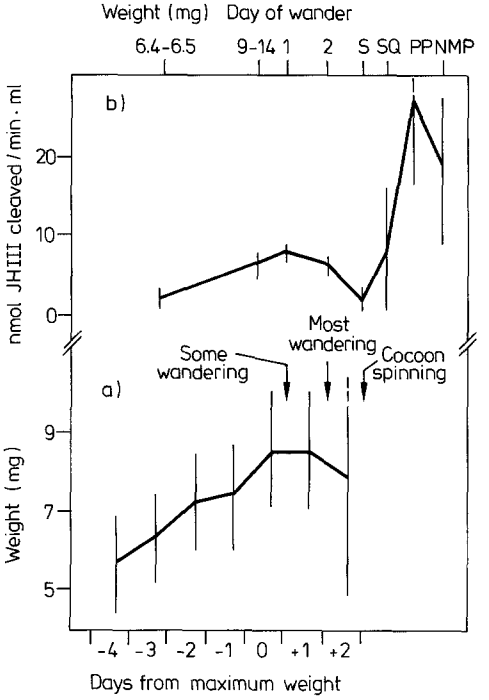

Fig. 4. a Weight gain and physiological markers observed during the last larval instar of *Ephestia elutella*. **b** Hemolymph JH esterase activity during the last instar (*S* cocoon spinning; *SQ* larva can squirm but not crawl; *PP* larva a stiff prepupa; *NMP* newly molted untanned pupa) (n=8 to 26)

Ephestia elutella (Hübner). The duration of and weight gain during much of the last larval instar are given in Fig. 4a. After cessation of feeding, larvae were not staged by weight but by the following developmental markers: Days 1 and 2 of wandering, on day 3 of wandering the larva spins a cocoon but retains the ability to walk, the larva can squirm but not crawl (Day 1 prepupa), larva is a stiff, motionless prepupa unable to curl (Day 2 prepupa), newly molted pupa.

The profile of JHE activity during the last larval instar is given in Fig. 4b. Two statistically significant peaks were found. The first peak occurred on the first day of wandering. The second peak, which had significantly greater activity than the first, occurred during the late prepupal stage. The decline of the second peak continued into the early pupal stage.

Estigmene acrea (Drury). The duration of and weight gain during the last larval instar are given in Fig. 5a. There was a significant sexual dimorphism between the maximum weights of females

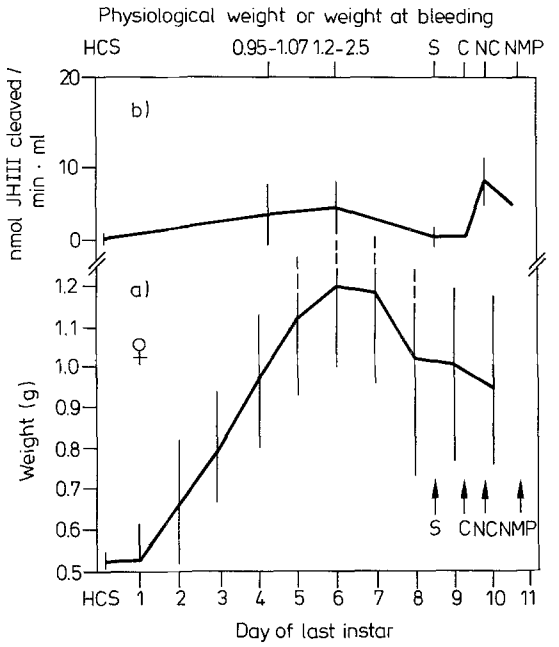

Fig. 5. a Weight gain and physiological markers observed during the last larval instar of *Estigmene acrea* (*HCS* head capsule slippage to the last instar; *S* cocoon spinning; *C* larva can curl but not walk; *NC* loss of ability to curl; *NMP* newly molted pupa). **b** Hemolymph JH esterase activity during the last instar ($n=4$ to 10)

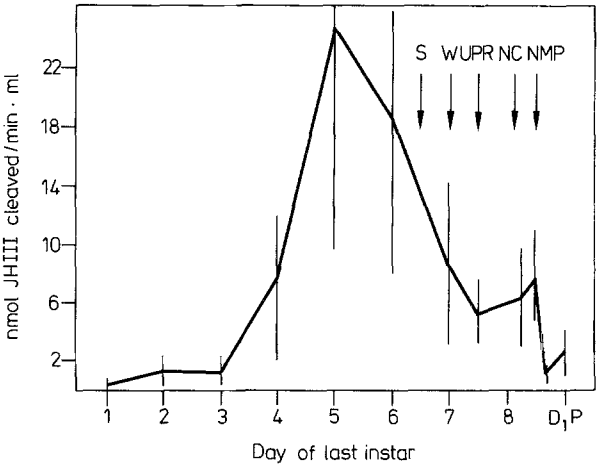

Fig. 6. JH esterase activity during the last larval instar and early pupa of *Galleria mellonella* (*S* first cocoon spinning; *W* dense cocoon built but larva can still walk; *UPR* larva cannot walk but can upright itself; *NC* no longer able to curl; *NMP* newly molted pupa; D_1P day 1 pupa) ($n=5$ to 12)

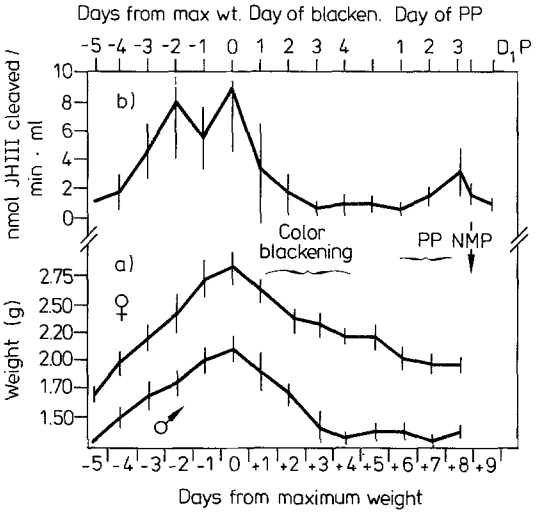

Fig. 7. a Weight gain and physiological markers observed during the last larval instar of male and female *Hemileuca nevadensis* (*PP* prepupa; *NMP* newly molted pupa). **b** Hemolymph JH esterase activity during the last instar (D_1P day 1 pupa) ($n=3$ to 23). JH esterase activity remained low through day 3 of the pupal stage

(1188 mg) and males (864 mg) (median test, $P<0.05$). Only females were bled for assay. After reaching maximum weight, larvae were synchronized with developmental markers. The markers used were: cocoon spinning, loss of ability to walk, loss of ability to curl, and newly molted pupa.

The profile of JHE activity during the last larval instar is given in Fig. 5b. Two statistically significant peaks were found. The first peak occurred at the time of maximum weight and the second peak at the loss of ability to walk. The heights of the two peaks were not significantly different.

Galleria mellonella (L.). This species was not monitored for weight gain during the last larval instar. Staging was done by day during the feeding stage and by physiological markers after feeding. Developmental markers used were: first cocoon spinning, dense cocoon with ability to walk, loss of ability to walk, loss of ability to curl, newly molted pupa (untanned), and tanning but still soft pupa (early day 1 pupa).

The profile of JHE activity during the last instar is given in Fig. 6. The first statistically significant peak found occurred on day 5 and activity then slowly declined back down as the pupal molt approached. The peak occurred over 24 h before the usual time of cocoon spinning. A second transient and more difficult to detect small JHE peak occurred at the pupal molt. Data used to define this peak were derived from bleedings from 3 separate generations. The duration of the peak is apparently much less than 24 h. The activity of the first peak was significantly greater than that of the second peak.

Hemileuca nevadensis (Stretch). The duration of and weight gain during the last larval instar are given in Fig. 7a. There was a high degree of sexual dimorphism in size of the last instar, the females

reaching a significantly greater maximum weight (2.8 g) than males (2.1 g) (median test $P<0.05$). To stage feeding larvae, only several µl of hemolymph were drawn for assay. The larvae were then reared to pupation to determine the time from bleeding until maximum weight. Larvae bled and reared in this manner usually had weight gains similar to unbled controls. After cessation of feeding, the following developmental markers were used to synchronize test larvae: color changes from bright yellow to dull yellow with increasing black (days 1, 2, 3, and 5 of this condition), days 1, 2, 3 of prepupa (prepupa defined as a postwandering larva which could not walk or curl so that the head touched the tail), newly molted soft pupa, sclerotized pupa (data only shown for day 1). No parasites emerged from any of these field collected larvae.

The profile of JHE activity during the last instar and early pupal stage is given in Fig. 7b. Two statistically significant peaks were observed. The first peak occurred at the time of maximum weight and the second peak during the late prepupa, shortly before ecdysis. The first peak was significantly greater than the second.

Manduca sexta (L.). During the feeding phase of the last larval instar, weight was primarily used to stage larvae. Toward the end of the feeding phase, the appearance of a white, chalky material on the feces ('frosted frass', Nijhout and Williams 1974) was used. After frosted frass, larvae were staged by exposure of the dorsal vessel, wandering, the day of the prepupal phase, metathoracic bars (Vince and Gilbert 1977) and newly molted pupa.

The profile of JHE activity during the last larval instar is given in Fig. 8. The two statistically significant peaks found occurred at the appearance of frosted frass and metathoracic bars, respectively. The activity during the first peak was significantly geater than during the second.

Orgyia vetusta (Boisduval). The weight gain and developmental markers observed during the last larval instar are given in Fig. 9a. There was a high degree of sexual dimorphism in maximum weight, the females weighing significantly more (525 mg) than the males (225 mg) at maximum weight (median test, $P<0.05$). Therefore, several µl of blood were taken from each larva, and these larvae were then reared through to determine time of bleeding prior to maximum weight or pupation. In most cases, development of these larvae followed the general profile of control larvae. All larvae were also observed to see which, if any, of these field collected larvae were parasitized. Toward the end of the instar, larvae were not staged by distance from maximum weight but by distance from pupation.

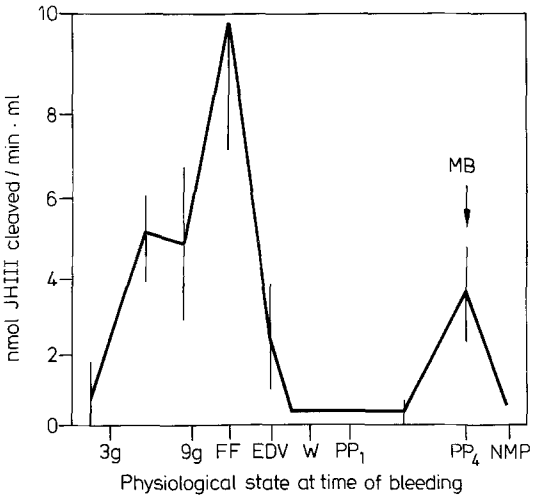

Fig. 8. Hemolymph JH esterase activity during the last larval instar of *Manduca sexta* (*g* gram weight; *FF* frosted frass; *EDV* exposure of dorsal vessel; *W* wandering; *PP* prepupa; *MB* metathoracic bars; *NMP* newly molted pupa) ($n=5$ to 12)

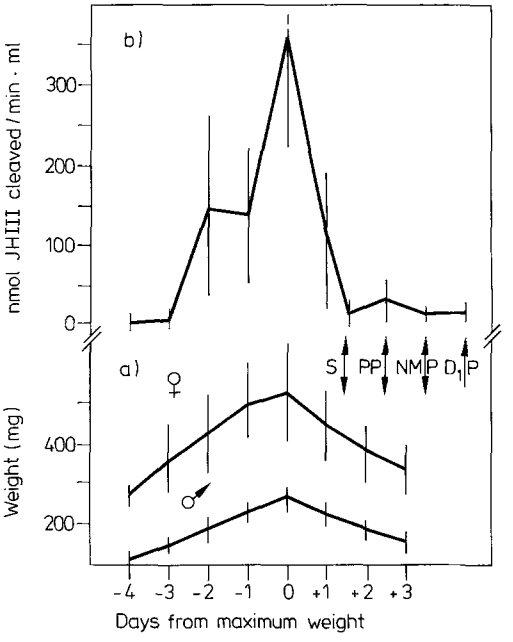

Fig. 9. a Weight gain and physiological markers observed during the last larval instar of male and female *Orgyia vetusta* (*S* cocoon spinning; *PP* prepupa; *NMP* newly molted pupa; D_1P day 1 pupa). **b** Hemolymph JH esterase activity during the last instar and early pupa ($n=2$ to 9). Esterase data from male and female larvae were combined. The second peak activity was 28.7 nmoles JH III cleaved/min vs 7.2 and 12.9 for adjacent time points

The profile of JHE activity during the last instar is given in Fig. 9b. Two statistically significant

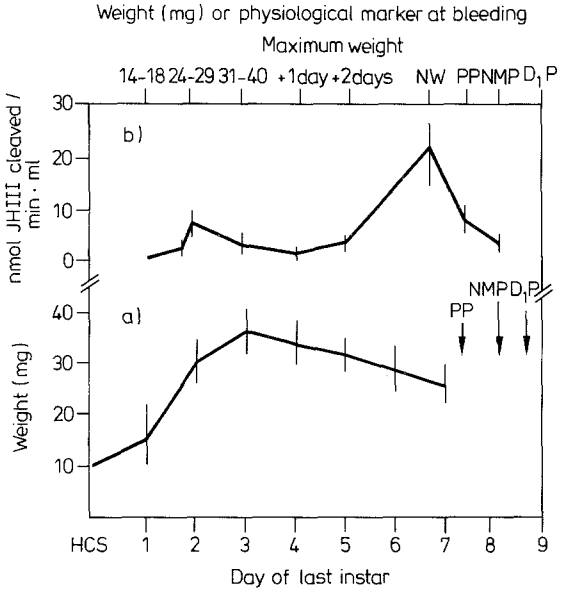

Fig. 10. a Weight gain and physiological markers observed during the last larval instar of *Pectinophora gossypiella* (*PP* larva a stiff prepupa; *NW* not able to walk; *NMP* newly molted pupa; D_1P day 1 pupa). **b** Hemolymph JH esterase activity during the last instar and early pupa ($n=4$ to 5)

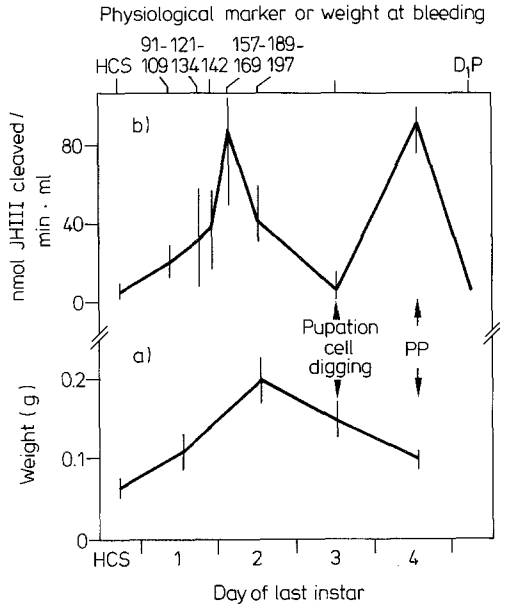

Fig. 11. a Weight gain and physiological markers observed during the last larval instar of *Spodoptera exigua* (*HCS* head capsule slippage of penultimate instar; *PP* larva is stiff prepupa; D_1P day 1 pupa. **b** Hemolymph JH esterase during the last instar ($n=3$ to 25). Weights are mg at top of **b**

peaks were found. The first and significantly greater peak occurred at the time of maximum weight and the second peak one day prior to pupation. The declining second peak continued on into the early pupal stage. Tachinid parasites emerged from 3 larvae bled at the time of host spinning and from 2 hosts bled as prepupae. The mean JHE activity in these hosts was almost exactly that of unparasitized hosts.

Pectinophora gossypiella (Saunders). The duration of and weight gain during the last larval instar are given in Fig. 10a. Post-feeding markers used to stage larvae included: 1 and 2 days post-maximum weight, loss of ability to walk, prepupa (defined as an immobile, stiff larva), newly molted pupa, sclerotized day 1 pupa.

The profile of JHE activity during the last larval instar and early pupal stage is given in Fig. 10b. Two statistically significant peaks of activity were found. The first peak occurred one day prior to maximum weight and the second peak occurred at the time of loss of the ability to walk. The second peak was significantly higher than the first.

Spodoptera exigua (Hühner). The duration of and weight gain during the last larval instar are given in Fig. 11a. After cessation of feeding, larvae were not staged by day but by several developmental markers. These markers were: digging of a pupation cell, prepupa (defined as loss of the ability to walk or curl), sclerotized day 1 pupa.

The profile of JHE activity during the last instar is given in Fig. 11b. Two statistically significant peaks were found. The first peak occurred shortly before maximum weight and the second peak occurred during the short prepupal stage. The heights of the first and second peaks were not statistically different.

Comparison of JHE Activity in Different Species

Table 2 gives a statistical comparison of the median JHE activities of the first and second peaks among the 11 species, plus 2 other species for which data was available. In general, the species could be placed in discrete groups by the amount of JHE activity. The first peak in *Orgyia vetusta* (388 nmoles/min·ml) was significantly higher than that in any other species. *Spodoptera exigua* and *Pseudoplusia includens* (Walker), with a first peak of 95.8 and 71.5, respectively, were not significantly different, although their activity was higher than in the remaining species. The next group was *Trichoplusia ni* and *Galleria mellonella* (38.1 and 20.7 nmoles/min·ml), while *Hemileuca nevadensis, Manduca sexta, Ephestia elutella, Junonia coenia,* and *Pectinophora gossypiella* had lower but very similar activities (5.9–8.3 nmoles/min·ml). The next group *Estigmene acrea* and *Pieris rapae* had

Table 2. Comparison of last larval instar hemolymph JH esterase activity at the first and second JHE peaks in 13 species of Lepidoptera[a]

Species	(Mean) median first peak nmoles/min·ml		JH titer 1st peak ng/ml (or g)	(Mean) median second peak nmoles/min·ml		JH titer 2nd peak ng/ml (or g)
Orgyia vetusta	(350.1)	388.0a		(28.1)	23.7cd	
Spodoptera exigua	(95.8)	95.8b	85[e]	(94.5)	98.4b	50[e]
Pseudoplusia includens[b]	(71.5)	71.5b		(126.0)	126.0a	
Trichoplusia ni[c]	(40.0)	38.1c		(31.4)	27.5c	
Galleria mellonella	(24.9)	20.7cd	1.9[e]	(8.2)	8.5d	0.044[e]
Hemileuca nevadensis	(8.9)	8.3de		(3.1)	2.6e	
Manduca sexta	(10.3)	8.2de	1.36[e]	(3.2)	3.2e	0.72[e]
Ephestia elutella	(8.1)	8.1de		(27.7)	29.1c	
Junonia coenia[d, f]	(6.9)	6.4e		(3.2)	3.8e	
Pectinophora gossypiella	(6.6)	5.9e		(22.2)	21.6cd	
Pieris rapae[f]	(4.5)	4.4ef	0.03[e]	(2.6)	2.2e	0.02[e]
Estigmene acrea	(4.2)	3.2f		(7.6)	6.6d	
Danaus plexippus[f]	(0.8)	0.8g		(0.5)	0.4f	

[a] Species medians followed by a different letter have significantly different peak JHE activity, nonparametric multiple comparison test

[b] Data on *Pseudoplusia includens (soybean looper)* from Sparks, personal communication using partition assay, JH I substrate

[c] Data for first peak obtained from study of Wing et al. (unpublished); for second peak from Jones et al. (unpublished), using partition assay, JH III substrate

[d] This species had no second peak. JHE activity is from prepupa shortly before ecdysis corresponding to the time of the second peak in the other species

[e] *Spodoptera exigua* from Yagi (1976) using data for *Spodoptera littura; Manduca sexta* from Fain and Riddiford (1975), Sridhara et al. (1978); *Pieris rapae* from Varjas et al. (1976) using data for *Pieris brassicae*; *Galleria mellonella* from Hsiao and Hsiao (1977)

[f] Butterflies. The remaining species are moths

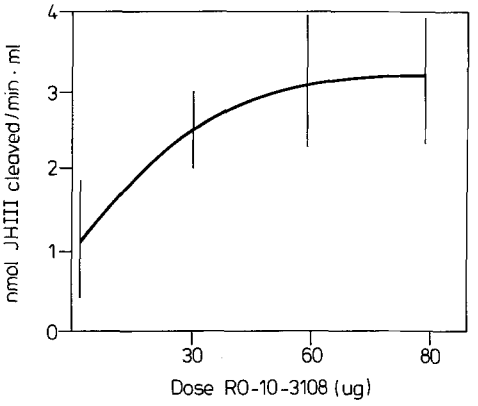

Fig. 12. Hemolymph JHE activity of day 1 pupae of *P. rapae* topically applied with various doses of the juvenoid Ro-10-3108 and bled 24 h later ($n=10$ individuals per data point)

very little activity (3.2–4.4 nmoles/min·ml) and the final species, *Danaus plexippus*, had extremely low activity (0.8 nmoles/min·ml).

The species groups listed above for the activity during the first peak were not the same groups as those for the second peak of activity. There was no relationship between the heights of the first and second peak (r_s not significant at 0.05 level).

Induction of Pupal JHE Activity in Pieris brassicae

It has been shown that JH or JH active juvenoids will induce JHE activity in the pupae of several species of moths. Since JHE activity in the butterflies was generally lower than that in moths, it was desirable to test whether juvenoid applications would induce JHE activity in butterfly pupae. Fig. 12 shows that JHE activity could be induced in a statistically significant dose-dependent manner by topical applications of the juvenoid Ro-10-3108 (Hoffman-La Roche). The absolute level of induced activity was much lower than that in juvenoid treated *Trichoplusia ni* pupae (Wing et al. 1981), as well as the fold induction (3 fold, vs 6 fold in *Trichoplusia ni*).

Discussion

There are several results of this study which enable conclusions to be drawn about JHE's in Lepidoptera. First, the occurrence of two significant peaks of JHE activity in the last larval instar is by far the most common situation. In many of these species the JH esterase activity is due to a single enzyme or to a group of very similar enzymes (Wing et al., in preparation). Only one species, *Junonia coenia*, was found to have a single peak of JHE activity during the last larval instar.

Second, there is great variability in the relative activities of the first and second peaks. In some species (*Manduca sexta, Hemileuca nevadensis, Galleria mellonella, Pieris rapae, Orgyia vetusta*),

the first peak is significantly higher than the second. In other species (*Trichoplusia ni, Spodoptera exigua, Danaus plexippus, Estigmene acrea*), the peaks demonstrated similar levels of JHE activity. Finally, in still other species (*Pectinophora gossypiella* and *Ephestia elutella*), the second peak is significantly higher than the first. It also appears that the butterflies, in general, have much lower hemolymph JHE activity than the moths, although JHE activity can be increased in the hemolymph pool by JH or JH analog applications in species from both groups.

Another possible generalization concerns the time of occurrence of the two peaks. No species possess a peak of JHE activity early in the last larval instar. Instead, there is a rise, variable in steepness, usually culminating in a first peak at or near the time of maximum weight. The timing of the first peak in *Samia cynthia* Drury, Saturniidae (Weirich and Wren 1976) also appears to be similarly placed. The JHE activity usually then declines back down to, or close to, the level occurring early in the instar. The second peak then occurs in the prepupal stage, shortly before the pupal molt. The second peak seems most closely correlated with the physiological marker of loss of the ability to walk or curl, which occurs shortly before pupation.

The species with just one peak, *Junonia coenia*, also begins the last larval instar with a low JHE activity and also shows a subsequent rise near the time of maximum weight. The time of peak activity, however, occurs midway through the prepupal stage, which is in between the time of the 2 peaks found in the other species. The activity then gradually declines back to the level of the early instar. Apparently, in this species one of the two last instar peaks that is missing. It should be noted that the JHE activity of *Galleria mellonella* published by Hwang-Hsu et al. (1977) differs from that found here in that the activity observed in their study did not decline after wandering and then show a prepupal rise. Instead, it declined to an intermediate level at which it hovered until pupation. In their study, larvae were bled at 24 h intervals and thus a small, transient rise in JHE activity at pupation may have gone undetected. Also, the maximum activity observed in this study for *Manduca sexta* and *Galleria mellonella* is lower than that previously published. Although this could be due to different assay procedures, or strain differences, we have found seasonal differences in *Trichoplusia ni* activity as did Vince and Gilbert (1977) for *Manduca sexta*.

In both *Manduca sexta* and *Galleria mellonella* the juvenile hormone titer declines to an extremely low level (Nijhout and Williams 1974; Hsiao and Hsiao 1977) concomitant with the first peak of JHE activity (Vince and Gilbert 1977; Hwang-Hsu et al. 1979). This JHE peak has been hypothesized to be necessary for eliminating residual hemolymph JH once the corpora allata have been inactivated. The great differences among the 13 species in Table 2 in the heights of the first JHE activity peak indicate that degradation may be a more important mode of JH clearance during the mid-instar in some species than in others.

The differences in magnitude of peaks of the activity prompt hypotheses concerning the endocrine events involved in lepidopteran metamorphosis. The galleria wax test has been used to measure the JH titer in *Manduca sexta, Galleria mellonella, Pieris brassicae* and *Spodoptera littura* (F.). The use of the same assay procedure facilitates comparison of the JH titers in each of these species. The titers in *Pieris brassicae* and *Spodoptera littura* are considered here as representative of the titers in *Pieris rapae* and *Spodoptera exigua*. The titer in *Galleria mellonella* was determined on a per gram tissue basis, while in the others the unit was per ml hemolymph. However, the JH concentration per gram of tissue in *Manduca sexta* is essentially the same as the concentration per ml hemolymph (Schooley, personal communication). Since the prepupal peak of *Trichoplusia ni* JHE activity appears to be induced by the prepupal burst of JH and can also be induced in moths and butterflies in a dose-dependent manner by topical applications of JH and juvenoids, it would be of interest to see whether the heights of prepupal peaks of JH and JHE in different species are related. The regression of JHE activity on JH concentration using the data in Table 2 (*Manduca sexta, Galleria mellonella, Pieris rapae* and *Spodoptera littura*) yields a statistically ($P=0.05$) significant and provocative rank correlation value ($r_s=0.80$).

The brain-subesophageal ganglion-controlled first peak of JHE activity is modulated but apparently not totally regulated by JH since JH or juvenoid application only slightly increases the first peak of esterase activity in *Manduca sexta* and *Trichoplusia ni* while allatectomy reduces but does not eliminate the peak (Sparks and Hammock 1979; Bhaskaran et al., Riddiford and Hammock and G. Jones, unpublished information). Nevertheless, this peak has been hypothesized to be important in clearance of JH so that PTTH-ecdysone release may proceed and metamorphosis begin. The regression of JHE activity on JH concentration (first peaks) using the data in Table 2 again yields a significant ($P<0.05$) and provocative rank correlation of $r_s=1.00$.

Another factor which could impinge on the induction of JHE by JH is the fraction of circulating JH which is bound to a carrier protein. Using the data of Wing et al. in preparation of the binding protein parameters in *Trichoplusia ni, Heliothis zea, Manduca sexta, Galleria mellonella* and *Estigmene acrea*, and a heuristic JH I titre of 10^{-8} M, the fraction of JH in bound form ranged from 83–99%. A weak trend for JHE activity to be negatively correlated with fraction of JH bound was observed, but no conclusions could be drawn.

In summary, then, it appears that most Lepidoptera have 2 last instar peaks of JHE activity, which vary in relative size from species to species. The peaks occur near the time of maximum weight and late in the prepupal stage. Finally, JH can induce JHE activity in at least pupae and probably prepupae of both moths and butterflies.

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