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JUVENILE HORMONE INVOLVEMENT IN PUPAL DIAPAUSE OF THE FLESH FLY *SARCOPHAGA CRASSIPALPIS*: REGULATION OF INFRADIAN CYCLES OF O_2 CONSUMPTION

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SUMMARY

1. A cyclic pattern of juvenile hormone (JH) activity is retained during pupal diapause in the flesh fly, *Sarcophaga crassipalpis*.

2. Cycles of JH activity correlate with infradian cycles of O_2 consumption. JH activity progressively increases during a 4-day cycle and appears to trigger the onset of an M_{O_2} peak.

3. During the first 2 days of an M_{O_2} cycle, pupae are insensitive to an application of JH analogue, but when JH analogue is applied during the last 2 days of the cycle, M_{O_2} rises and the cyclic M_{O_2} pattern is destroyed. When JH analogue is applied to third instar larvae, O_2 consumption is sustained at a steady, high rate throughout pupal diapause.

4. The M_{O_2} cycles persist in abdomen-ligated pupae but disappear following head ligation.

INTRODUCTION

Pupal diapause is characterized by a shut-down of the brain-prothoracic gland system in saturniid silkmoths (Williams, 1946, 1947, 1952). This model for diapause is also applicable to flesh flies (Fraenkel & Hsiao, 1968; Ohtaki & Takahashi, 1972; Žďárek & Denlinger, 1975; Gibbs, 1967; Walker & Denlinger, 1980), but in addition, diapause in flesh flies is associated with a unique juvenile hormone (JH) profile (Walker & Denlinger, 1980). At puparium formation, flies destined for continuous development lack JH activity while flies programmed for pupal diapause show major pulses of JH activity having a periodicity of 24 h. In this study we extend our observations on the JH titre beyond the onset of diapause and suggest a link between infradian cycles of oxygen consumption (Denlinger, Willis & Fraenkel, 1972) and JH activity.

MATERIALS AND METHODS

Insect rearing

Cultures of *Sarcophaga crassipalpis* were reared as previously described (Denlinger, 1972). To induce pupal diapause, adult flies lacking a diapause history were

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maintained at 25 ± 1 °C, 12L: 12D (light: dark cycle), and their progeny were maintained at 20 ± 0·5 °C, 12L: 12D. With these conditions diapause incidence exceeded 99 %. Pupal age was carefully defined by collecting newly formed puparia at hourly intervals.

Measurement of O_2 consumption

Oxygen consumption (M_{O_2}) of individual pupae was monitored at 25 °C using a Scholander respirometer (Mark Co., Brockton, MA). Diapausing pupae, 10–30 days after pupariation, were transferred to a temperature of 25 °C for at least 2 days before making the initial recording. Pupae were kept in the respirometer continuously and manometric measurements were recorded at 24-h intervals. The infradian cycles of O_2 consumption in this species have a periodicity of about 4 days at 25 °C (Denlinger *et al.* 1972).

JH extraction and bioassay

JH was extracted from pooled, whole body homogenates (21 g, approx. 175 pupae) using the procedure of Hsiao & Hsiao (1977). Haemolymph JH determinations were based on 5 ml samples pooled from larvae at the time of pupariation (0 h) or collected 8 h after pupariation. Activity was determined using the *Galleria* wax wound bioassay (deWilde *et al.* 1968; deLoof & van de Veire, 1972) and scoring was based on the response of 10–12 pupae tested at each dilution. Activity in whole body extracts was expressed in *Galleria* units (GU) per gram fresh weight and activity in haemolymph samples as GU ml^{-1} haemolymph. One GU corresponds to 5 pg JHI.

Application of JH analogue

The juvenile hormone analogue methoprene (ZR515) kindly provided by Zoecon Corporation (Palo Alto, CA) was applied directly to the head of diapausing pupae. Solvents could not be used as hormone carriers since many organic solvents are highly active in terminating diapause (Denlinger, Campbell & Bradfield, 1980). To deliver a dose of approximately 50 μg/pupa, 0·5 mg of the analogue was distributed among 10 pupae. Since solvents do not interfere with diapause when applied prior to pupariation, JHA applied to third instar larvae was dissolved in 5 μl acetone.

Ligation

Pupae were neck ligated by placing a fine cotton thread around the neck and puncturing the head to permit tightening of the ligature. Remnants of the head were then cut off and remaining fluid was absorbed with a filter paper. In a similar manner, abdomen ligations were performed by puncturing the tip of the abdomen and tightening the ligature at mid-abdomen.

RESULTS

Haemolymph JH

Previous observation of JH activity in diapausing flesh fly pupae was based on activity extracted from whole body homogenates (Walker & Denlinger, 1980). T

determine whether such JH is merely sequestered within the corpora allata (contained within the ring gland) or is indeed released into the haemolymph, a 5 ml haemolymph sample from newly pupariated flies (0 h) programmed for pupal diapause was compared to haemolymph collected from flies 8 h beyond pupariation. Whole body homogenates showed high activity (1000 GU g^{-1}) at 0 h and no detectable activity 8 h after pupariation (Walker & Denlinger, 1980). Activity in the haemolymph reflected a similar pattern. Though some activity (10 GU ml^{-1}) was detected in pupae collected at 8 h, JH activity in haemolymph at 0 h was considerably higher (2600 GU ml^{-1}).

JH activity in whole body homogenates

The earlier report of JH activity in diapause-destined flesh flies was limited to the first 4 days following puparium formation. Data included in Fig. 1 extend the results until day 12. JH activity is prevalent throughout this interval, and daily cycles noted during the first 4 days persist for several additional days. But, the activity patterns

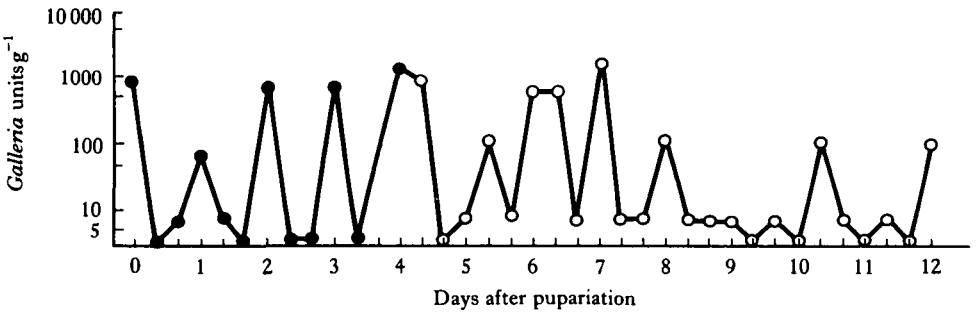

Fig. 1. Juvenile hormone titre in *Sarcophaga crassipalpis* programmed for pupal diapause (20 °C, 12L: 12D). Solid circles are data from Walker & Denlinger (1980).

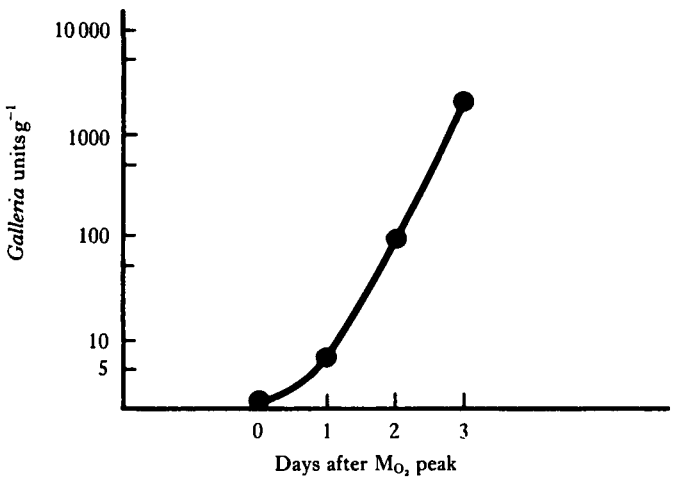

Fig. 2. Juvenile hormone titre in *Sarcophaga crassipalpis* on different days of an infradian oxygen consumption cycle at 25 °C.

Table 1. *Elimination of O_2 consumption cycles in diapausing pupae of* Sarcophaga crassipalpis *by topical application of a juvenile hormone analogue (50 μg ZR515) at selected times during an O_2 consumption cycle or before pupariation*

Time of JHA application	No. of pupae	Elimination of M_{O_2} cycles (%)
During M_{O_2} cycles		
untreated controls	49	0
day of M_{O_2} peak	14	14·2[a]
1 day after peak	10	20·0[a]
2 days after peak	10	80·0[b]
3 days after peak	14	78·0[b]
Before pupariation		
Acetone controls (5 μl)	10	0
JHA	10	100

Values followed by the same letter are not significantly different at the 5 % level (Chi square).

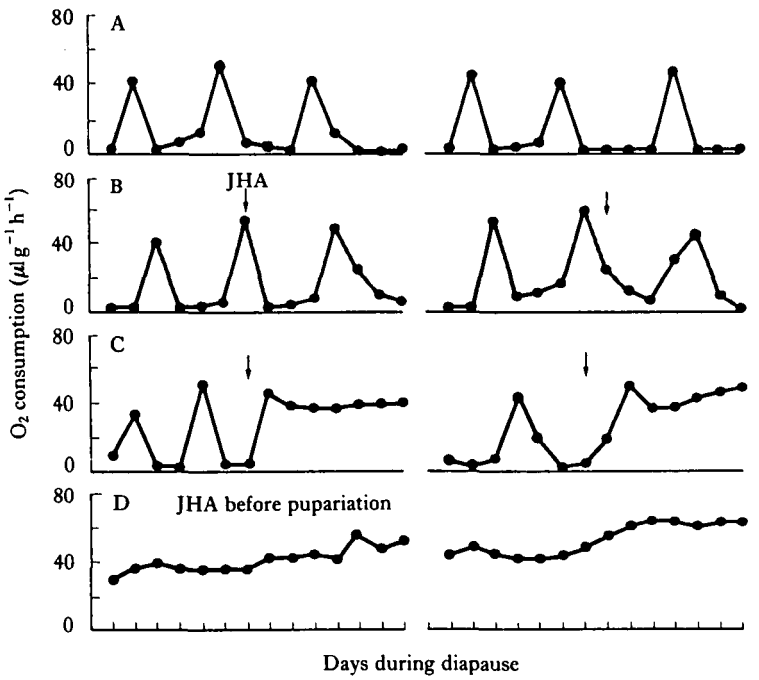

Fig. 3. Patterns of oxygen consumption in representative diapausing pupae of *Sarcophaga crassipalpis* that have been (A) untreated, or received a topical application of JH analogue (B) on the day of an M_{O_2} peak or 1 day later, (C) 2 or 3 days after an M_{O_2} peak, or (D) before puparium formation. Arrow indicates day of JH analogue application.

become much less precise in older samples. Since the samples consisted of a pool of approximately 175 pupae, the reduction in cycle precision could be caused by a gradual loss of synchrony among pupae, a genuine alteration of cycle periodicity, or a combination of these two events.

JH activity in relation to M_{O_2} cycles

In flesh flies, O_2 is not consumed at a steady rate during diapause: days of high O_2 consumption occur with a periodicity of about 4 days in *S. crassipalpis* at 25 °C, and during other days O_2 consumption is barely detectable (Denlinger *et al.* 1972 and Fig. 3A). To determine if cycles of JH activity may be linked to the metabolic cycles, diapausing pupae were assayed for JH activity on different days of the O_2 consumption cycle. As shown in Fig. 2, JH activity was undetectable in pupae collected on the peak day of O_2 consumption but increased progressively toward the approach of the next M_{O_2} peak.

Altering M_{O_2} cycles with a JH analogue

The JH analogue ZR515 was applied topically to pupae on different days of the M_{O_2} cycle to test its ability to alter the cycle. When applied on the day of peak O_2 consumption or 1 day later, JHA had little effect on the subsequent metabolic cycle (Table 1 and representative responses shown in Fig. 3B). By contrast, JHA application 2 or 3 days after an M_{O_2} peak (1 or 2 days before the next anticipated peak) ended the cyclic pattern and caused pupae to shift to a sustained pattern of high metabolic activity (Fig. 3C).

JHA applied to third instar larvae just prior to pupariation did not alter the decision to enter diapause, but the pupae failed to exhibit the normal cycles of O_2 consumption (Table 1). In such pupae, O_2 consumption remained at a steady, high rate throughout

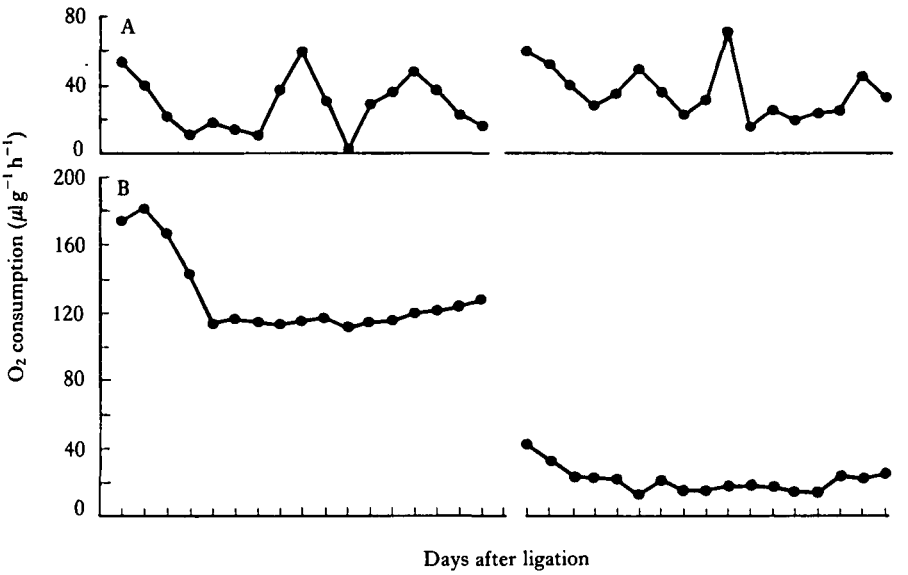

Fig. 4. Patterns of oxygen consumption in representative diapausing pupae of *Sarcophaga crassipalpis* that have been (A) abdomen ligated or (B) neck ligated.

the duration of diapause (Fig. 3D). The elevated metabolic rates elicited by JHA treatment were very similar to rates observed during a normal M_{O_2} peak ($40–60\,\mu l\,g^{-1}\,h^{-1}$).

Effect of neck ligation on M_{O_2} cycles

If JH is involved in regulation of the M_{O_2} cycles, removal of the corpora allata, the site of JH synthesis, should eliminate the cycles. The corpora allata of fly pupae are contained within the ring gland and selective surgery is thus extremely difficult. As a less selective alternative to allatectomy, a neck ligature was used to produce a headless pupa. In a control group, a ligature was tied at mid-abdomen, and the posterior region, roughly equal to the head volume, was destroyed.

With both types of ligation, M_{O_2} was initially high and then progressively decreased during the following 2–4 days (Fig. 4A, B). The abdomen-ligated pupae then reverted to a cyclic M_{O_2} pattern (Fig. 4A, 90 % retained cyclic patterns, $N = 10$), although the cycles were more erratic and the metabolic rates were generally higher than among intact pupae (Fig. 3A). In contrast, the cyclic M_{O_2} pattern was halted by neck ligation (Fig. 4B, 0 % retained cyclic M_{O_2} patterns, $N = 10$). Though M_{O_2} cycles were consistently eliminated in all pupae by neck ligation, the level at which M_{O_2} was sustained was variable as shown by the two representative individuals in Fig. 4B: 6 of the 10 pupae maintained a low M_{O_2} of $15–40\,\mu l\,g^{-1}\,h^{-1}$ while the other 4 pupae stabilized at a much higher rate ($100–150\,\mu l\,g^{-1}\,h^{-1}$).

DISCUSSION

This study with flesh flies documents the presence of JH, not only at the very onset of pupal diapause (Walker & Denlinger, 1980), but also later in diapause. The precise 24-h cycles of JH activity noted at the onset of diapause, however, become much less distinct in older pupae. Since activity was determined using pooled samples, the

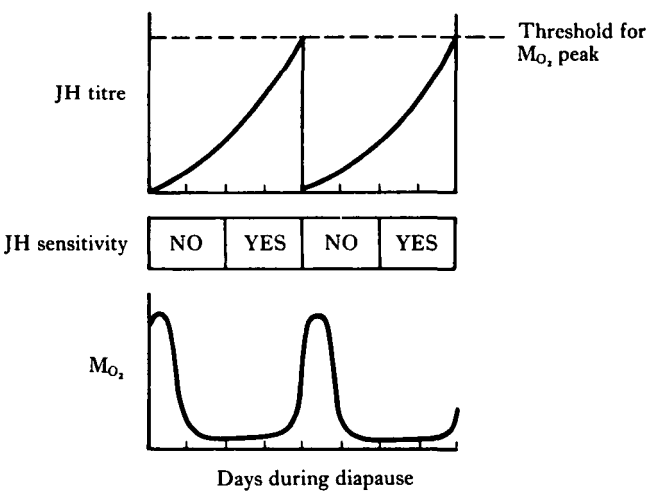

Fig. 5. Model of the relationship between JH titre, JH sensitivity and M_{O_2} cycles during pupal diapause in *Sarcophaga crassipalpis*.

apparent dampening of the cycle may represent individual pupae becoming less synchronous with time. Alternatively, this effect could be caused by the transition to a different JH activity pattern as diapause progresses. We suspect that both events are occurring.

Detection of JH activity in the haemolymph, as well as in whole body homogenates, implies that the hormone is not merely being retained within the corpora allata but is circulating and thus available to function physiologically. We suggest that JH is involved in regulating the metabolic cycles that persist throughout diapause.

In flesh flies, the rate of O_2 consumption during diapause is not constant (Denlinger *et al.* 1972). Days of high O_2 consumption are separated by several days in which O_2 consumption is barely detectable. At 25 °C, the periodicity of *S. crassipalpis* is about 4 days, but at lower temperatures the periodicity is greater. At lower temperatures, it is also apparent that cycle periodicity changes during diapause: early in diapause, peaks of activity are close together; in mid-diapause, the periodicity is increased; and as the end of diapause is approached, the cycles again become closer together. By focusing on the 4-day cycles observed at 25 °C, we find a close correlation between JH activity and phase of the O_2 consumption cycle. We suggest that JH activity progressively increases during the phase of low O_2 consumption, reaches a critical threshold, and initiates a rise in O_2 consumption (Fig. 5). During the M_{O_2} peak, JH activity declines sharply and cannot be detected by bioassay techniques.

Diapausing pupae are highly sensitive to JH late in the M_{O_2} cycle. If a pupa is supplemented with exogenous JH shortly before an M_{O_2} peak, the M_{O_2} rises to a normal peak but fails to return to the base level. By contrast, application of JHA during an M_{O_2} peak or 1 day later fails to elicit an effect, suggesting that at this time the hormone is either degraded very rapidly or JH receptors are not present. When JHA is applied before pupariation, the M_{O_2} cycles never appear. Pupae are locked into a sustained pattern of high M_{O_2} (40–60 $\mu l\, g^{-1}\, h^{-1}$). While this rate is comparable to that observed on days of M_{O_2} peaks, it remains considerable lower than the nadir (150 $\mu l\, g^{-1}\, h^{-1}$) of non-diapausing pupae (Denlinger *et al.* 1972).

The high M_{O_2} stimulated by JHA may account for the efficacy of JHA in shortening diapause. When applied to third instar larvae, JHA can reduce the length of diapause by half (Denlinger, 1981). This suggestion, however, implies that diapause termination is hastened by the utilization of a finite energy reserve. Though such a mechanism is possible, its existence has not yet been demonstrated. The presence of JH during diapause may also produce a covert, cumulative effect on the other neuroendocrine centres that would eventually result in activation of the brain-prothoracic gland system. This, too, could account for the shortening of diapause observed when pupae are supplemented with extra JH.

Corpora allata and corpora cardiaca have been implicated in control of the metabolic rate in several species. Allatectomy lowers M_{O_2} in adults of *Calliphora erythrocephala* (Thomsen, 1949), *Leucophaea maderae* (Sägesser, 1960), and *Pyrrhocoris apterus* (Sláma, 1964), but in diapausing prepupae of *Monema flavescens*, allatectomy elevates M_{O_2} (Takeda, 1978). In *P. apterus*, M_{O_2} is further depressed when the corpus cardiacum is also removed (Sláma, 1964). Allatectomy fails to alter M_{O_2} in *Blaberus discoidalis*, but extirpation of the corpora cardiaca significantly lowers M_{O_2} (Keeley & Friedman, 1967). In the *Blaberus* cockroaches, the corpora cardiaca are the source

of a neurohormone that enhances respiratory capacity of fat body mitochondria by stimulating synthesis of cytochromes aa_3+b (Keeley, 1981). Many effects of hormones on respiration may not be direct. The M_{O_2} response we observe in flesh flies could be secondary to another metabolic change elicited by JH.

The difficulty of extirpating corpora allata from the ring gland of fly pupae precludes the surgical manipulations that are possible in some other species. But, from our head ligation experiments it is clear that the metabolic cycles are dependent upon a cephalic regulatory mechanism. Headless pupae become acyclic. In contrast, cycles persisted when a portion of the abdomen was ligated. The level of O_2 uptake observed in pupae following head ligation may be a function of the phase of the M_{O_2} cycle at the time of ligation, but this possibility has not been tested.

Whether JH is involved in the pupal diapause of other insects remains unclear. Histological evidence suggests that the corpora allata of *Mimas tiliae* remain active during pupal diapause (Highnam, 1958), and slight JH activity is detectable in *Hyalophora cecropia* early in diapause (Gilbert & Schneiderman, 1961). By contrast, there is no evidence to suggest a role for the corpora allata during the pupal diapause of *Manduca sexta* (Bradfield & Denlinger, 1980). The M_{O_2} cycles are not unique to the pupal diapause of *Sarcophaga* but have also been reported in several species of Lepidoptera (Crozier, 1979). Such species are perhaps most likely to share a similar regulatory mechanism utilizing JH during diapause. As with larval diapause (Chippendale, 1977; Beck, 1980), the role of JH in pupal diapause may be highly variable among different species.

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