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THE PHYSIOLOGY OF WANDERING BEHAVIOUR IN *MANDUCA SEXTA*

I. TEMPORAL ORGANIZATION AND THE INFLUENCE OF THE INTERNAL AND EXTERNAL ENVIRONMENTS

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SUMMARY

1. A stereotyped series of behavioural changes occurs in preparation for pupation in *Manduca sexta*. Feeding declines over an 8-h period, after which the larva coats its body with a labial gland secretion. The animal then begins a 10- to 30-h wandering behaviour during which it constructs a pupation chamber underground.

2. Wandering behaviour starts during a specific temporal gate which is determined by an internal circadian timer. The scotophase of the day prior to wandering has the major influence on the timing of internal processes which activate the behaviour.

3. Wandering duration is correlated with larval size, reflecting a possible influence of juvenile hormone.

4. The larva appears to be irrevocably committed to begin wandering by an event that occurs about 15 h previously, a time that corresponds to the second of three prothoracicotropic hormone (PTTH) pulses and the accompanying elevation of ecdysteroids as measured by Gilbert *et al.* (1981).

5. We conclude that both the initiation and duration of wandering behaviour are governed primarily by processes which are internal to the larva.

INTRODUCTION

In preparation for pupation many insect larvae exhibit specialized patterns of behaviour which secure a protective microhabitat in which pupation can occur. Thus, elaborate cocoons are spun by some insects, whereas others, such as the tobacco hornworm, *Manduca sexta*, enter a wandering phase and burrow into the soil to construct an underground chamber for pupation.

Such premetamorphic behaviour is often highly stereotyped and occurs at a specific developmental stage, thereby allowing a detailed analysis of the roles of internal (e.g. organs, hormones, etc.) and external (e.g. substrates, photoperiods, etc.) factors which may influence the behaviour. For example, the duration and organization of

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cocoon spinning of saturniid silkworms are regulated primarily by internal rather than external factors (Van der Kloot & Williams, 1953*a*,*b*; Lounibos, 1975, 1976) and appear to be activated by the endocrine environment which precedes pupation (Bounhiol, 1938; Riddiford, 1972; Lounibos, 1976). By contrast, various other types of insect behaviour, such as nest provisioning in several species of solitary wasps (Fabre, 1880; Peckham & Peckham, 1898; Steiner, 1962), strictly require external (visual) cues for normal performance. As a foundation for the analysis of the endocrine and neural factors that regulate wandering behaviour in *Manduca*, this paper first describes the temporal organization and then identifies the principal components of the internal and external environments which influence its expression.

MATERIALS AND METHODS

Experimental animals

Larvae of the tobacco hornworm, *Manduca sexta*, were raised in individual containers on an artificial diet (Bell & Joachim, 1976) at 25 ± 1 °C under either short day (12L: 12D) or long day (17L: 7D) photoperiods in which lights-off is designated as 00.00 AZT (arbitrary 'zeitgeber' time; Pittendrigh, 1965). Under these conditions larvae which ecdysed to the fifth instar during the scotophase at the start of day 0 began wandering during the scotophase of either day 4 or day 5 and were designated as Gate I and Gate II respectively (Truman & Riddiford, 1974*a*; Nijhout & Williams, 1974*a*).

Behavioural studies

The onset and duration of wandering locomotion were routinely monitored in tilting Petri dish actographs (Fig. 1A). Each larva was placed in a plastic Petri dish which would tilt on its copper axle when the animal crawled around the circumference of the dish, thus closing an electrical contact which was recorded on an Esterline Angus event recorder.

Wandering locomotion of individual larvae was also recorded in a large container filled with soil which allowed undisturbed behaviour in a natural substrate (Fig. 1B). Vibrations caused by the animal's movements in the soil were recorded from a Plexiglas rod with a piezo-electric transducer (phonograph cartridge), and were amplified and recorded on a Gould chart recorder. Background vibrations were eliminated by conducting these observations on a seismically-free, concrete, geophysics pier.

Burrowing and cell building movements were observed directly by placing the larva in a narrow (2 cm) Plexiglas chamber (17×26 cm) filled with soil, similar to a child's 'ant farm'. The number of pupation chambers excavated and the movements involved could be readily seen.

The time of cessation of feeding for each particular larva was determined by measuring the change in weight of its piece of diet over a 2-h interval prior to wandering. Following these feeding measurements the larva was placed in an actograph to determine the interval between the midpoint of the measured feeding interval and the onset of wandering.

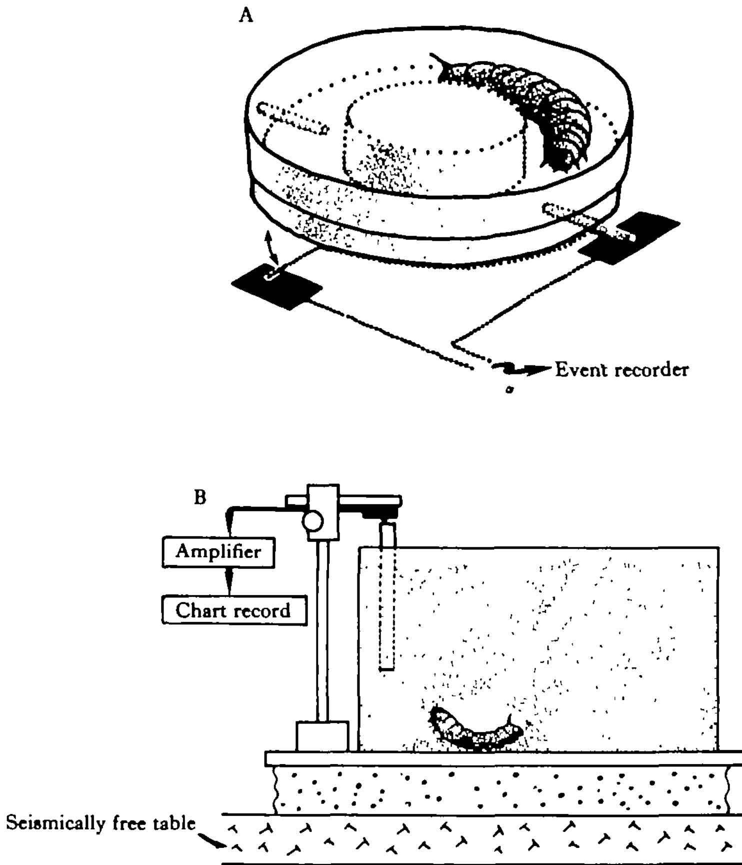

Fig. 1. Devices for monitoring wandering locomotion. (A) Tilting Petri dish actograph. Arrow marks direction of tilt as larvae crawls around circumference of chamber. Electrical contacts are between wire (· · · · ·) and copper plates (black). (B) Method for recording vibrations of larva under soil. Piezo-electric transducer (black) mounted on micromanipulator detects vibrations in soil *via* Plexiglas rod.

Surgical treatments

Larvae were anaesthetized with CO_2 for several minutes prior to any surgery. Mandibular glands were removed several hours before the start of wandering under modified Weevers' saline (Truman, 1978*b*) by gently pulling them with No. 5 forceps through a small (5–7 mm) ventral incision in the third thoracic segment. The incision was closed with a suture (Tycron 5-0). In control larvae the mandibular glands were not removed during the surgery.

Release of fluid from the midgut was prevented by ligating the anal flap with cotton thread and then sealing the anus with melted beeswax (Lounibos, 1976) several hours prior to wandering. Conversely, anaesthetized larvae were 'milked' after 8–10 h of wandering to cause early fluid release. Control larvae were ligated and waxed on their anal prolegs or they were squeezed without expelling fluid.

Photoperiod manipulations

The photoperiodic control of wandering was studied using tilting actographs placed in light-tight boxes in a constant temperature room (24 ± 1 °C). Photoperiods were maintained in these boxes with fluorescent lamps programmed by an automatic timer (Tork). Food was also placed in the actograph with larvae which were still feeding.

RESULTS

Behavioural changes

Termination of feeding

The first behavioural manifestation of the preparation for wandering was the cessation of feeding. Based on time-lapse videotape analysis of the behaviour of larvae, Reinecke, Buckner & Grugel (1980) concluded that feeding abruptly ceased in *Manduca* about 8 h prior to wandering. However, food consumption as measured by changes in diet weight rapidly decreased starting at about this time, but persisted at a low level until 1·5–2 h before the onset of sustained locomotion (Fig. 2). This suggests that feeding is not terminated suddenly, but rather declines over the 8-h period before wandering starts.

Body coating behaviour

At the end of feeding, the larva coated its body with a viscous proteinaceous labial gland secretion (Hakim, 1976), termed 'body wetting' (Reinecke *et al.* 1980). The

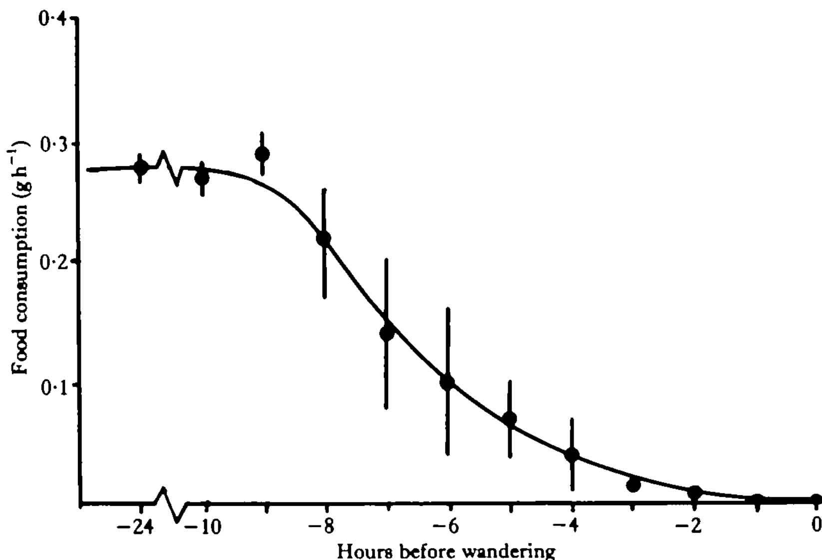

Fig. 2. Decline of feeding activity. Food was presented for 2 h at various times before the onset of wandering, and the change in weight of food during this time was measured (mean ± S.D.; N = 4–18 per point). Curve fitted by eye.

larva first applied the secretion for 15–30 min along one side of its body, anterior to posterior, and then along the other side. The cuticle was left temporarily moist and glistening. Subsequent coating movements occurred intermittently until, after a series of halting but increasingly complete abdominal peristaltic waves, the larva began crawling persistently, which marked the start of wandering behaviour. The function of the proteinaceous coating applied to the cuticle during this period has not been determined, but it may serve as a dry lubricant for burrowing (Hakim, 1976). In any case, coating the body is not a prerequisite for normal wandering, for larvae confined in tubes could coat only the tube walls in front of them, and yet they initiated wandering after the same 80 ± 30 min interval (mean ± S.D.; $N = 5$) as unconfined larvae.

The dorsal vessel became apparent during body coating, followed later by the appearance of a variable amount of reddish ommochrome pigment (Hori & Riddiford, 1982), and the gut was purged of remaining faecal pellets. In *Manduca* the purging of the gut involves the voiding of relatively dry faecal pellets, rather than the very liquid material expelled at this time by saturniid larvae (Lounibos, 1975).

Wandering behaviour

In fifth instar *Manduca* larvae, the persistent locomotion which characterizes wandering began suddenly at the end of the body coating period. Within groups of larvae which ecdysed synchronously to the fifth instar at 25 °C under short (12L: 12D) or

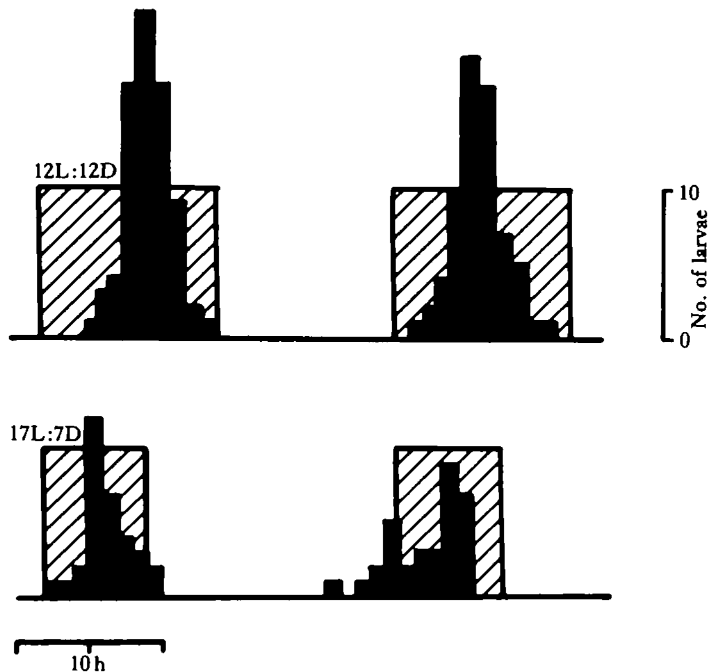

Fig. 3. Histograms of the time of onset of wandering behaviour under short day (12L: 12D) and long day (17L: 7D) photoperiods. Cross hatching represents the scotophases of the 4th and 5th days after the start of the last instar. Larvae wandering on the night of day 4 are called Gate I larvae; those on day 5 are Gate II larvae. Onset times were recorded with tilting dish actographs.

long (17L: 7D) day photoperiods, the onset of wandering occurred during a specific interval or 'gate' (Pittendrigh, 1966) in the photoperiodic cycle: approximately half of the larvae initiated wandering during the fourth night following ecdysis (Gate I larvae) whereas the remaining larvae wandered on the following night as Gate II larvae (Truman & Riddiford, 1974*b*; Nijhout & Williams, 1974*a*). In larvae maintained under either long day or short day photoperiods, wandering began during an 8- to 9-h gate which encompassed most of the dark period (Fig. 3). Under the respective regimens, Gate II larvae began wandering 1·5–2·5 h earlier relative to lights-off than did Gate I larvae. Thus the gating of wandering behaviour shows a 'gating bias' as has been reported for the gating of adult eclosion of *Drosophila* (Pittendrigh & Skopik, 1970) and prothoracicotropic hormone (PTTH) release in *Manduca* (Truman, 1972). About 30% of the long day Gate II larvae began wandering prior to the start of darkness, which indicates that the decision to initiate wandering is not precipitated by the onset of the scotophase on the night of wandering.

Wandering larvae showed three primary types of behaviour: crawling, burrowing and cell building. Crawling consisted of stereotyped waves of muscle contraction which were initiated at the terminal abdominal segment and passed anteriorly from segment to segment in a characteristic sequence of segmental lifting and promotion (Barth, 1937). A wandering larva can crawl for hundreds of feet, but when provided with an appropriate substrate, it begins burrowing even at the very beginning of the wandering period (Reinecke *et al.* 1980; personal observation). As observed in narrow Plexiglas 'ant farms', burrowing behaviour was characterized by repeated thrusts and flexions of the thorax and head while the abdomen was held rigid and stationary. When a small space had been formed in front of the animal as a result of this thoracic shovelling, the larva took a single step forward and then repeated the process. Within the soil the larva used a slightly different series of movements to construct a pupation chamber. Initially it tunnelled in tight circles to excavate the chamber, and then it slowly moved about within the chamber, compacting the cell walls with strong dorsal and lateral flexions of the head and thorax. The larva smoothed the interior of the chamber with its mandibles and eventually impregnated the walls with fluid stored in the gut and expelled principally from the mouth when soil was present.

Activity during the wandering phase was monitored primarily in tilting dish actographs. Records (e.g. Fig. 4) showed an abrupt onset of persistent spontaneous locomotor activity which continued for 17·5 ± 5 h (mean ± s.d.; $N = 37$) for Gate I larvae and 20·5 ± 6 h ($N = 45$) for Gate II animals at 23 °C. Visual observation of larvae in these situations showed that during the first 12 h of wandering, crawling and burrowing movements predominated. The slow turns, head extensions and mandibular scraping patterns typical of cell building became increasingly frequent after 12 h. Release of impregnation fluid was from the anus when there was no soil present and began at 14–16 h after the onset of wandering, continuing for many hours (Reinecke *et al.* 1980).

The average stepping frequency of spontaneous crawling changed characteristically during wandering (Fig. 5). The frequency increased over the first 4 h of activity and then maintained a peak level of about 0·9 steps s^{-1} until, at 12 h, a gradual decline in stepping rate began. The initial reduction of stepping rate coincided with the time

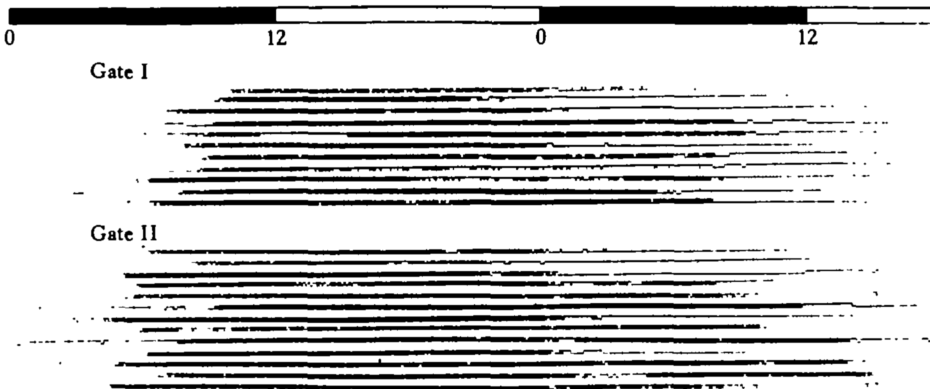

Fig. 4. Actograph records of wandering behaviour in short day larvae. Each strip represents the locomotor record of an individual larva. The photoperiod is represented at the top of the figure as dark (scotophase) and light (photophase) bars beginning on the night when wandering starts for each gate.

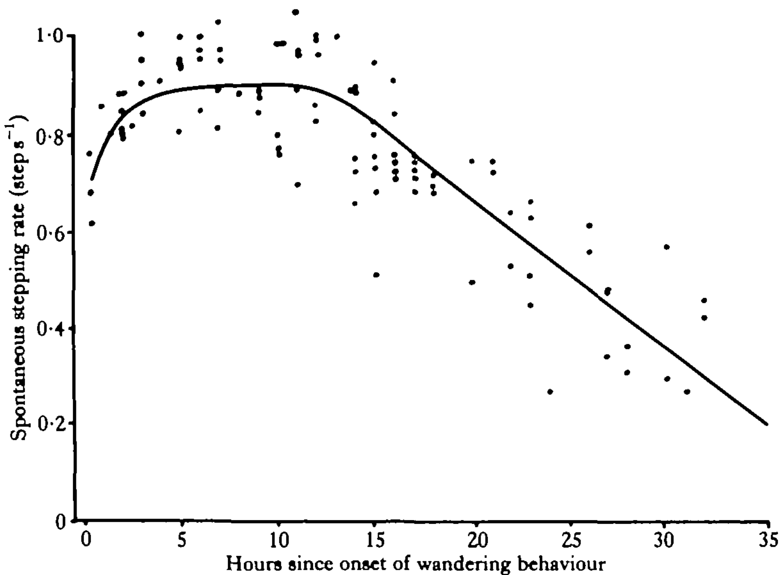

Fig. 5. Changes in crawling activity during wandering. The rate of stepping was determined by visual observation of the movement cycle of the terminal segment as the anal prolegs were placed on the substrate, raised and replaced. Each point represents the mean stepping rate of a different individual and is based on approximately five 30-s observations.

when cell building movements became increasingly common, suggesting that these changes may be inherent transitions in the behaviour pattern.

Following the cessation of the spontaneous locomotor activity of wandering, larvae were not motionless, but exhibited repeated head extensions until 42–44 h after wandering began. During this period the larva would reinitiate sustained locomotion for a variable period if disturbed.

Because the actographs represented an abnormal environment for the larva, wandering was also monitored in a more normal situation, a large container filled with soil.

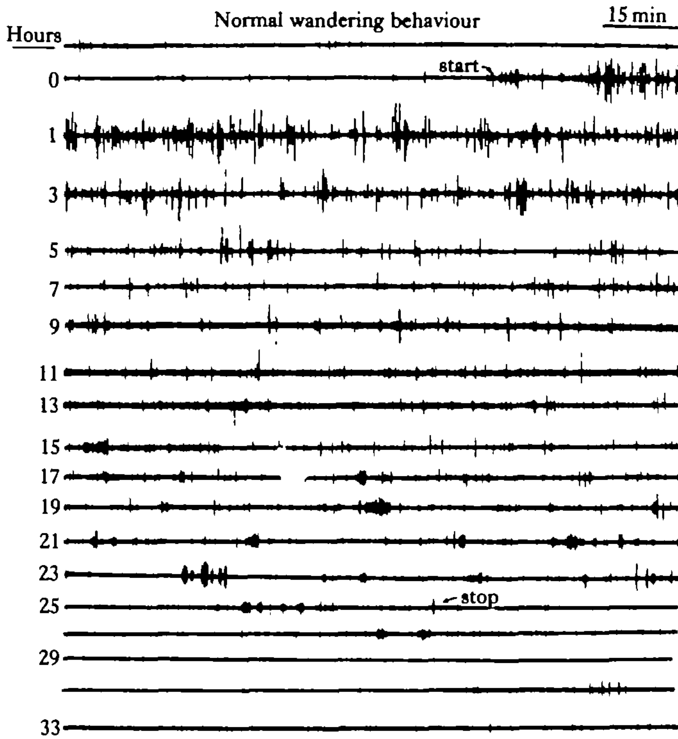

Fig. 6. Vibration record of wandering behaviour. The strips represent consecutive 2-h vibration records obtained from an individual larva as shown in Fig. 1B, starting about 3 h before the onset of wandering. The onset ('start') and termination ('stop') of wandering are marked on the record. The numbers to the left of the records mark elapsed time from the start of wandering.

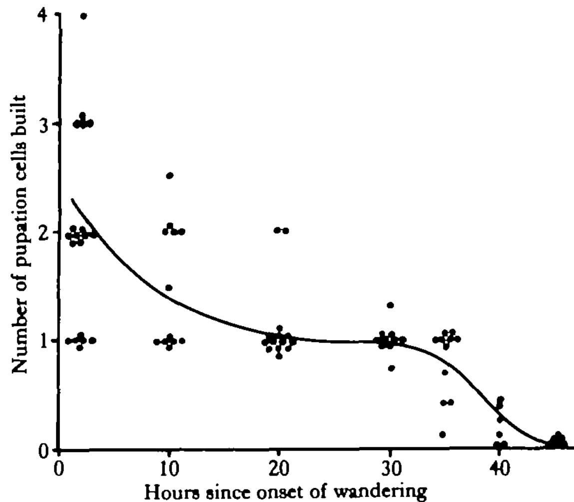

Fig. 7. Changes in number of pupation chambers constructed. Larvae were placed in narrow Plexiglas soil chambers at 0, 10, 20, 30, 35, 40 or 45 h after the start of wandering. After 48 h, the containers were opened and the number of pupation cells in each was counted. Fractional values were assigned when a clear but incomplete excavation was observed.

The soil vibrations generated by larval movements were monitored continuously with a piezo-electric transducer as described in Materials and Methods. As seen in the example in Fig. 6, locomotor movements appeared suddenly and persisted for 25 h after which time the larva became quiescent. For five larvae observed this way at 22 °C, the mean duration of wandering was 24 ± 4 h, a value similar to that observed in the actographs. In every case the larva formed a single pupation chamber at the bottom of the container (a depth of 20 cm).

Larvae were also placed in narrow Plexiglas containers filled with soil and were watched during burrowing and cell building behaviour. These animals typically constructed several pupation cells. The tendency of larvae to make multiple cells was a function of the length of time that they had been wandering prior to the time that they were placed on the soil (Fig. 7). Early in the period of wandering, the activity of the larva was powerful enough that the cell wall could be breached accidentally, even when it had been strengthened by several hours of packing. This then initiated a new period of tunnelling and excavation. Multiple cells were most common when larvae were placed in the observation chambers during the first 10 h of wandering.

By 20 h, the intensity of activity had decreased enough that larvae very rarely broke out of a cell during construction, so that only a single chamber was constructed. Importantly, spontaneous wandering activity in the actographs also ceased after about 20 h but if such animals were then placed on soil, they resumed activity and attempted to construct a pupation cell.

At 30 h, larvae began to lose their ability to complete even a single cell, and by 35–40 h they were often able to excavate only a slight depression at the soil surface. The ability of sensory input to prolong wandering also disappeared gradually between 30–48 h.

These results indicate that the early excavation of a pupation cell does not cause an early decrease in wandering intensity. Indeed, larvae placed in a preconstructed chamber at the outset of wandering broke out of the chamber and wandered as usual. In addition, wandering could not be prolonged beyond 48 h under normal circumstances, even when no pupation cell had been constructed.

Internal factors controlling wandering

Larval size

In *Manduca* last instar larvae must reach a critical size of about 5 g to initiate the decline in juvenile hormone which leads to metamorphosis (Nijhout & Williams, 1974*a*,*b*; Nijhout, 1975*a*,*b*). Thus, the fastest growing animals pass this threshold and subsequently wander during the first gate whereas slower larva initiate wandering during the second gate, as was first reported for the initiation of metamorphosis by Nijhout & Williams (1974*a*).

As described earlier, the duration of spontaneous wandering behaviour was essentially constant under a variety of substrate conditions ranging from unobstructed soil to plastic Petri dishes. However, there was a systematic variation in the duration of locomotion seen in Gate I *versus* Gate II larvae. The difference in the two groups of larvae reflects the fact that Gate I larvae are generally smaller at wandering than are Gate II animals. The duration of spontaneous locomotion shown by these larvae is strongly correlated with the animal's size (Fig. 8).

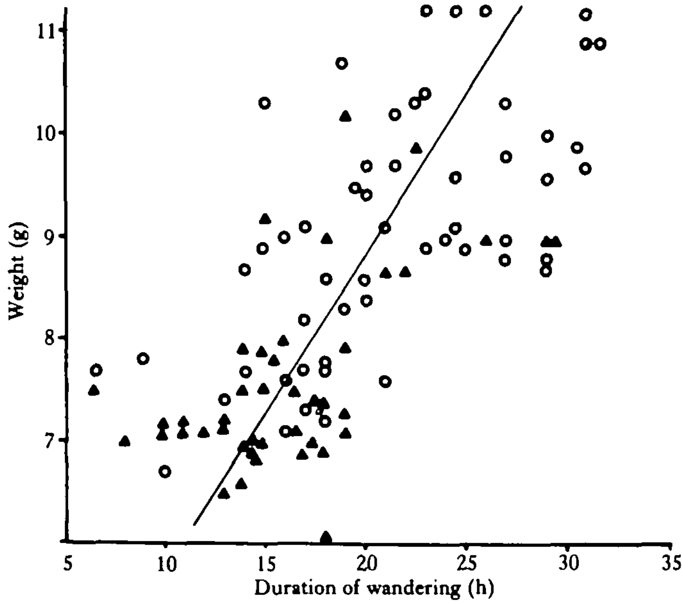

Fig. 8. Relation between weight and wandering duration. Wandering was measured in tilting dish actographs for larvae weighed at 18.00 AZT on the day prior to PTTH release. Filled triangles represent Gate I larvae ($r = 0.33$; $P = 0.01$); open circles represent Gate II larvae ($r = 0.37$; $P = 0.01$).

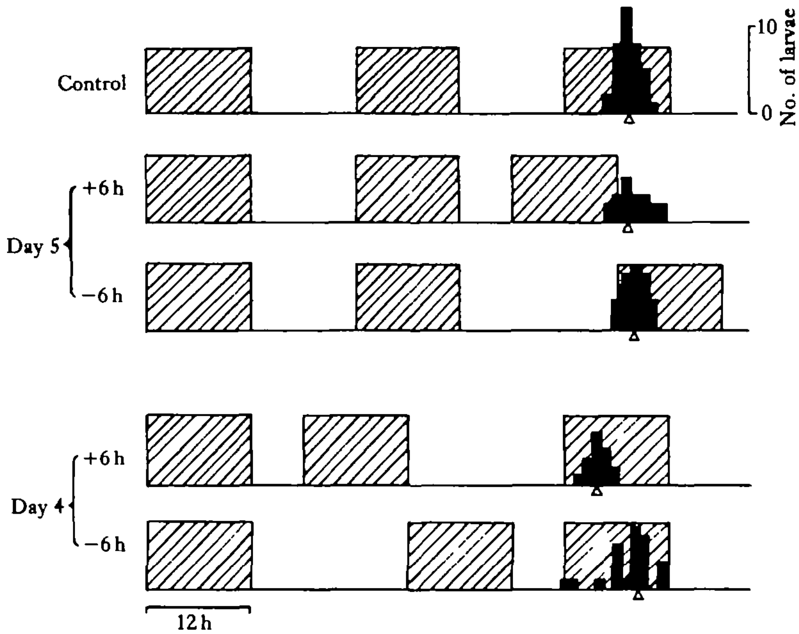

Fig. 9. Effect of scotophase shifts on the wandering gate in short day, Gate II larvae. Histograms represent the onset time of wandering recorded in tilting dish actographs. The direction of the scotophase shift and the day on which it occurred are noted at the left of the figure. The open triangles mark the mean onset time for each experimental group. Scotophase of 12L:12D photoperiod is indicated by cross-hatching.

Effects of manipulating the labial glands and fluid release

In the saturniid silkworms, the labial glands and the process of gut fluid release contribute to the control of cocoon spinning behaviour (Van der Kloot & Williams, 1953*b*; Lounibos, 1976). In *Manduca*, however, surgical removal of the labial glands 10–15 h prior to the onset of wandering altered neither the time of onset nor the duration of the behaviour ($N = 8$). Similarly, when fluid release was prevented by ligating and waxing the terminal segment closed ($N = 5$) or when it was advanced by gently 'milking' larvae ($N = 10$) after 10 h of wandering, wandering behaviour was no different from that of sham ligated and milked larvae. Thus, it appears that sensory feedback related to the accumulation in or release of material from the labial glands and gut does not play a role in regulating wandering behaviour in *Manduca*.

Role of photoperiod in controlling wandering

The importance of photoperiod in the control of wandering behaviour was examined by shifting various parts of the photoperiod and determining the resultant

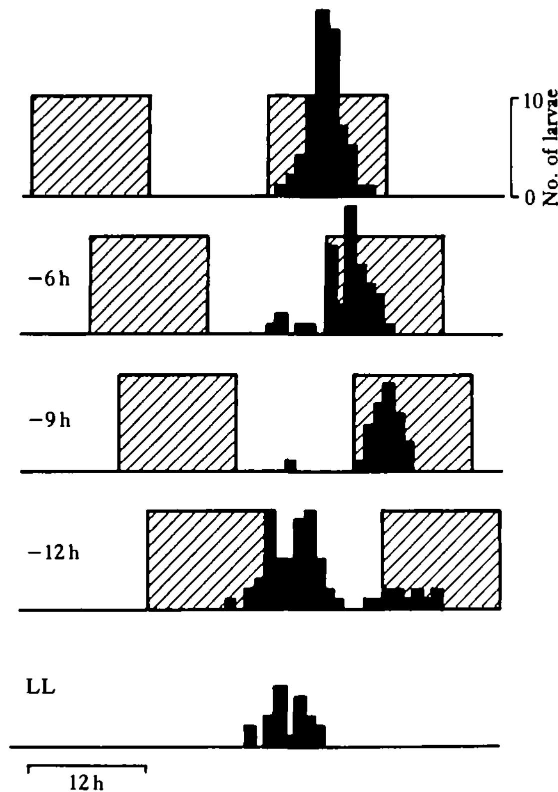

Fig. 10. Effects of delaying the day 4 scotophase on the onset of Gate II wandering. Starting time of wandering was recorded in tilting dish actographs and is shown as a histogram for groups of larvae in which the scotophase of day 4 was delayed by 0 (Control), 6, 9 or 12 h, or which were held in constant light (LL) starting on day 4.

effect on the onset of wandering. Fig. 9 shows the effect of a simple advance or del[ay] in the scotophase. When done on the night when wandering normally occurs, no change was seen in response to either a 6 h advance or a 6 h delay. By contrast, a 6 h advance or delay of the scotophase on the preceding night resulted in a marked advance or delay in the start of the behaviour. These results indicate that the scotophase on the night prior to wandering strongly influences the gating of the onset of wandering.

The role of the scotophase prior to wandering was examined more closely by systematically retarding it for Gate II larvae. Nearly the entire sample of larvae delayed the onset of wandering in response to a 9 h delay in the scotophase (Fig. 10), but when the delay of the scotophase was increased to 12 h, only 22 % delayed wandering. The remaining 78 % of the animals wandered several hours earlier than controls as though no scotophase had occurred. Thus, within this 3 h period a majority of the animals apparently had undergone a change which irrevocably determined the time at which they would begin wandering. The interval between this event and the start of wandering was about 15 h.

The timing of the event which determines the onset of wandering was further

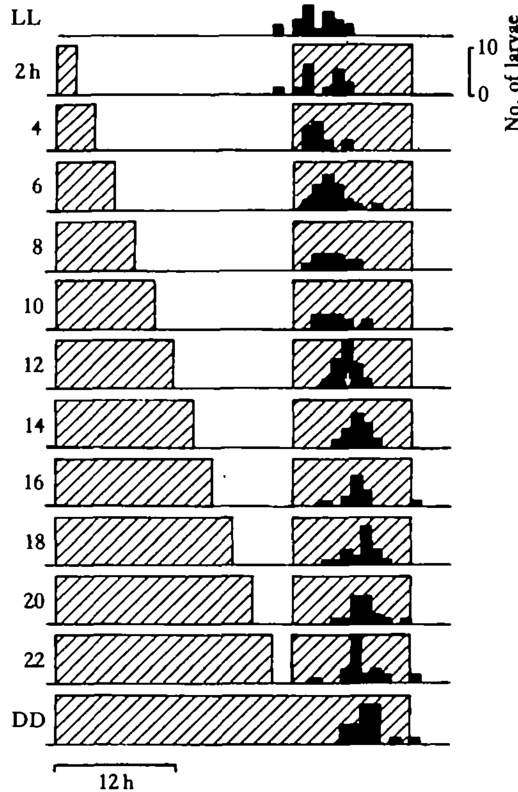

Fig. 11. Effect of changing day 4 scotophase length on Gate II wandering onset. Histograms indicate onset of wandering, recorded in tilting dish actographs, of short day Gate II larvae exposed on day 4 to a scotophase which varied in length from 0 (LL) to 36 h, as noted on the left of the figure. The white arrow marks the mean wandering onset time for control larvae in a 12L: 12D photoperiod.

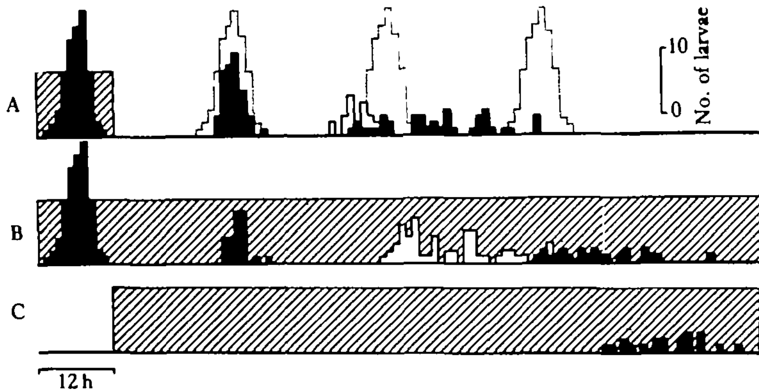

Fig. 12. Distribution of the onset of wandering under constant conditions of light or darkness. Histograms represent distributions of larvae in 12L: 12D up to time of treatment. Distribution of wandering onset times observed under 12L: 12D is shown as black histograms at left, while the predicted repetitions of this distribution under 12L: 12D are represented as the broken line histograms. (A) Larvae were exposed to constant light starting at 12.00 AZT of day 1 (black histograms on right), day 2 (white histograms) and day 3 (grid histograms) of the fifth instar. (B) Larvae were exposed to constant darkness starting at 00.00 AZT of day 1 (black on right), day 2 (white), day 3 (grid). (C) Shifted larvae were exposed to constant darkness beginning at 12.00 AZT on day 1, 12 h later than for cohorts (black histogram in B).

examined by systematically changing the length of the scotophase on the night before wandering. Scotophases shorter than 4 h were without effect and the animals began wandering several hours early as if they had been kept in continuous light (Fig. 11). This lack of effectiveness of very short scotophases has been observed in other systems (Lees, 1971; Truman, 1972). Continued lengthening of the dark period beyond 4 h resulted in a systematic shift in the onset of wandering up through dark period lengths of 14–16 h. Scotoperiods longer than this caused no further shift in the time of wandering. In these experiments, alteration of the dark period became ineffective in shifting wandering about 15 h prior to the actual onset of behaviour. These results agree with those of the previous experiment: the onset of wandering is influenced by a photoperiod-sensitive event which occurs about 15 h prior to the start of the behaviour.

The possible involvement of a circadian rhythm in the control over wandering was examined by maintaining short day larvae in constant light or constant darkness. As seen in Fig. 12A, exposure of larvae to constant light (LL) resulted in good synchrony of wandering on the first and second cycle (with a slight advancement for the latter) followed by randomized wandering onset after three LL cycles. This arrhythmia induced by LL is seen in a number of insects (Pittendrigh, 1966; Truman, 1971*b*,*c*).

The behaviour of *Manduca* in constant darkness (DD) also indicated a rapid decay of the rhythm into randomness. Three complete cycles in DD are shown in Fig. 12B. The first cycle shows good synchrony but the next two distributions are each spread over a 24-h period. This type of randomized wandering could be due to a true arrhythmia or to an excessive variability in the free-running periods of each larva. These possibilities were tested by delaying the start of DD on day 1 by 12 h for one group. When these larvae wandered 3 days later, the distribution of onset of wandering was also delayed by 12 h (Fig. 12C). These data suggest that wandering is under circadian control but that the clocks of individual larvae have very different periods.

DISCUSSION

The central control of wandering and its associated behaviour

The behavioural transition from feeding to wandering consists of a stereotyped sequence of behaviour beginning with the decline and cessation of feeding, the body coating process, and the locomotor period which we will refer to specifically as wandering. Wandering itself follows a sequence of changes in intensity and motor pattern as cell building, fluid release, and quiescence eventually occur.

This sequence of behaviour during wandering does not vary substantially under differing external environmental conditions which include various substrates and photoperiods. Furthermore, each component of the sequence can occur independently of the normal performance of previous steps. Such adherence to a behavioural sequence without regard for either the external environment or the adequacy of previous efforts suggests that the sequence is internally organized.

Similar predetermined, internally sequenced behaviour has been described for the ecdysis of *Manduca* pupae (Truman, Taghert & Reynolds, 1980) and for the adult eclosion of several moths (Truman, 1971*a*), as well as for the prepupal cocoon spinning of the silkmoths (Van der Kloot & Williams, 1953*a*,*b*; Lounibos, 1976). In silkworms, the expulsion of material from the labial glands (Van der Kloot & Williams, 1953*b*) and the release of impregnation fluid from the gut (Lounibos, 1976) constitute internal cues which influence the timing of behavioural changes during cocoon spinning, but these do not play a role in regulating the wandering behaviour of *Manduca*. The correlation between larval size and duration (Fig. 8) indicates that the variability in this behavioural programme originates in internal processes probably related to the physiology of larval growth and development.

The external environment influences certain aspects of the behaviour pattern. The larva uses a variety of motor patterns in the process of crawling, burrowing and cell construction in response to a heterogenous external environment with its various obstacles and potential traps. Furthermore, as the interval of spontaneous wandering reaches its normal end, external disturbances, such as the loose soil in an unfinished pupation chamber, will evoke activity until the source of disturbance has disappeared. Indeed, continuous disturbance causes wandering to persist several days longer than usual and prevents subsequent pupal development (B. Cymborowski, personal communication).

Thus, the features of wandering which are regulated internally are the initiation time and the sequence of behaviour, the pattern of changes in locomotor intensity and the minimal duration of the behaviour. The external environment strongly influences the specific behaviour patterns used to cope with environmental heterogeneity (e.g. crawling, burrowing, etc.), and it can cause wandering to be prolonged or temporarily resumed after the programmed minimal duration has expired.

The role of photoperiod in the control of wandering

The combined influences of a circadian clock and of processes related to larval size determine the initiation of wandering behaviour. The clock determines *when* the wandering gate will occur whereas size determines *which* gate will be utilized. The circadian nature of the clock is indicated by the observation that the rhythm o

re-run under conditions of DD. The broadening of the wandering distribution after several cycles in DD (Fig. 12B) appears to be due to variability in the free-running period of individual larvae. This obscures the underlying rhythmicity, but phase shift experiments (Fig. 12C) strongly suggest such rhythmicity to be present.

Under a photoperiod regimen, the time of the wandering gate is most strongly influenced by the scotophase on the night before wandering begins (Fig. 9). Both the lights-off signal which initiates this scotophase and the lights-on signal which terminates it influence the time when the behaviour starts (Figs 10, 11) in a manner similar to the hourglass model initially proposed by Truman (1971*c*) for the timer governing eclosion in *Antherea pernyi*. Consistent with the results obtained in silkmoths (Truman, 1971*c*) and aphids (Lees, 1971), the appearance of light during the first 4 h of darkness appears to prevent activation of a new cycle in *Manduca*. For several hours thereafter, the lights-on signal alters the kinetics of the timer, thereby adjusting the time when the gate opens.

The various experiments manipulating the scotophase indicate that the animals become committed to wander at a particular time by an event which occurs about 15 h earlier (Figs 10, 11). Several endocrine changes correlate with the time when the photoperiod-sensitive event occurs. PTTH release is known to be photoperiodically controlled in pupae of *Antherea pernyi* (Williams & Adkisson, 1964) and is gated in both the larval moulting cycle of *Manduca* (Truman, 1972; Fain & Riddiford, 1975) and prior to the onset of wandering (Truman & Riddiford, 1974*b*; Gilbert *et al.* 1981). This latter PTTH release induces the secretion of ecdysone which induces the changes of epidermal pigmentation (Nijhout, 1976) and of commitment for cuticle production (Riddiford, 1978) associated with wandering. On this basis, PTTH and ecdysteroids have been proposed as the trigger for wandering behaviour itself (Truman & Riddiford, 1974*a*, 1977; Truman, 1978*a*). Under long day (17L: 7D) photoperiods, the gated release of PTTH prior to wandering consists of three discrete pulses. The first pulse occurs 6 h after the start of the scotophase and the second is at about 12.00 AZT (Gilbert *et al.* 1981), which is 15 h before the wandering gate. This temporal relationship suggests that the second period of PTTH release and the accompanying elevation of ecdysteroids may be the photoperiod-sensitive event which results in wandering. The endocrine regulation of the onset of wandering will be considered in a subsequent paper in this series.

The role of size in the control of wandering

The gated release of PTTH which precipitates the sequence of events leading to pupation in the last instar of *Manduca* is thought to occur only when juvenile hormone (JH) has been removed from the haemolymph and its cellular effects sufficiently erased, a process which is initiated when the larva reaches the critical size of 5 g (Nijhout & Williams, 1974*a*,*b*; Nijhout, 1975*a*). Presumably, during the first gate, the largest larvae would be the first to complete the elimination of JH effects and thus the first to release PTTH. While the gate remains open, smaller larvae would subsequently release PTTH after they had finished the appropriate size-correlated processes. After the gate closes, the remaining larvae could complete the necessary processes to become competent to release PTTH so that hormone secretion would be possible as soon as the gate opened on the next day.

As described in the Results, the duration of wandering differed for Gate I and Gate II animals. But Gate I animals were, on average, smaller when they wandered than were Gate II larvae. When durations were compared for larvae of equal size (Fig. 8), there were no differences between the two gates. Instead, larvae in each gate showed the same proportionality between size and wandering duration. Since size is correlated with the decline of JH, this suggests that persistent JH-related effects may influence the behaviour pattern. This possibility that the larva's endocrine history relative to its JH titres may quantitatively alter the wandering programme will be explored in subsequent papers.

In summary the wandering behaviour of *Manduca sexta* is regulated by a combination of processes internal and external to the larva. Certain aspects of this behaviour, such as the time of onset of wandering and the minimal duration, are relatively stereotyped and inflexible owing to the large degree of internal control of these characteristics. Other aspects of wandering behaviour are highly responsive to variation in the external environment, such as locomotor pattern and prolonging wandering in response to disturbance, allowing an important degree of behavioural plasticity. Thus wandering behaviour consists of the interaction of stereotyped and plastic behavioural components determined by the internal and external environments.

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