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PHYSIOLOGY OF INSECT ECDYSIS

I. THE ECLOSION BEHAVIOUR OF SATURNIID MOTHS AND ITS HORMONAL RELEASE

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At the end of the last larval instar the mature silkworm larva stops feeding and spins a silken cocoon. In this chamber the animal subsequently pupates and in some cases overwinters in a dormant condition – termed diapause. Under the influence of the warmth and lengthening days of spring, the pupa terminates diapause and initiates adult development. Weeks later the mature moth sheds the pupal exuviae and escapes from the cocoon. In Cecropia (*Hyalophora cecropia*) and many other saturniids the larva has prepared for this eventuality by spinning into the cocoon an 'escape-hatch' or valve through which the moth emerges (Van der Kloot & Williams, 1953). Among the silkworms of the genus *Antheraea* the moth resorts to chemistry to escape from its stout-walled and valveless cocoon (Kafatos & Williams, 1964). A few days prior to emergence the moth secretes a crystalline enzyme, cocoonase, on to the surface of its maxillary galeae. Then, during the process of eclosion, buffer produced in the labial glands is also secreted on to the galeal surface. The resulting protease solution then digests the sericin 'glue' from around the silk fibres and thus provides an opening for the moth. After struggling free, the moth clings to the outside of the cocoon and spreads its wings.

The time of adult eclosion is dictated by a 'biological clock' which is located in the brain of the moth (Truman & Riddiford, 1970). At the prescribed time an 'eclosion hormone' is released from the corpora cardiaca and acts on the nervous system to trigger eclosion. The present report describes the behaviour involved in the escape of the moth from its pupal exuviae and cocoon. Also discussed is the role of the eclosion hormone in this control.

MATERIALS AND METHODS

1. *Experimental animals*

Pupae of *Hyalophora cecropia* and *Antheraea polyphemus* were obtained from dealers or reared outdoors on netted trees (Telfer, 1967). Cocoons of *A. pernyi* were obtained from Japanese sources. The pupae were stored at 5 °C for at least 12 weeks; they were then returned to 25 °C to initiate adult development.

2. *Recording apparatus*

The activity of pharate and developing adult moths was recorded as described by Truman & Riddiford (1970). Pupae were removed from the cold and a thread was waxed to the tip of the abdomen of each. Then, 1 or 2 days prior to the emergence of the adult, the thread from the animal was attached to a lever writing on a revolving smoked drum. Since lepidopteran pupae and developing adults are only capable of moving their abdomens, the above apparatus recorded essentially the total motor activity of the animals.

3. *Surgical procedures*

Operations were performed on either diapausing pupae or animals during the first few days of adult development. The surgical techniques and specific details of brain removal and brain transplantation were as described by Williams (1946, 1959). The other operations involved the same general techniques and were always performed through an opening in the facial integument. Extirpated organs were examined under a dissecting microscope to assure their complete removal.

In some experiments the abdomen of the pharate moth was isolated by clamping the first abdominal segment with a haemostat (Lockshin, 1969). The body of the moth was then cut away anterior to the clamp.

4. *Preparation and injection of homogenates with 'eclosion hormone' activity*

(A) *Preparation of homogenates*

By injection of a homogenate prepared from the brain of a pharate moth one can trigger the premature eclosion of another pharate adult (Truman & Riddiford, 1970). In the present experiments active homogenates were prepared by removing the brain and corpora cardiaca from developing moths which were within 3 days of eclosion. The tissues were homogenized with a ground glass homogenizer in 20 μl of Ringer's solution (Ephrussi & Beadle, 1936). Homogenates were then either used immediately or frozen until needed. Each homogenate was injected through the mesothoracic tergum of the pharate moth by means of a 50 μl Hamilton syringe.

(B) *Time of injection*

Under mormal circumstances the release of the eclosion hormone is dictated by a 'biological clock' (Truman & Riddiford, 1970); consequently, the time of injection is of paramount importance if one is to avoid false positive results. All experiments reported here were performed on silkmoths which had developed in a 17L:7D photoperiod regimen (photophase from 08.00 to 01.00 E.D.T.). Under these conditions Pernyi moths eclose only during the period from 19.30 to 24.30 and Cecropia only from 09.00 to 16.30. The pharate moths which were to be injected had completely digested the pupal endocuticle and were in advanced stages of resorption of the moulting fluid. Such individuals were injected with active homogenates 8–12 h before their particular eclosion gate.

RESULTS

1. *The pre-eclosion behaviour*

The movements of five Pernyi were monitored continuously from the time the pupae were placed at room temperature until they emerged as adult moths. The animals initiated adult development about 7 days after removal from the cold and emerged 20 days later. Up to and including the 14th day of adult development (according to the timetable given by Williams & Adkisson, 1964), the pattern of activity was extremely simple (Fig. 1 *A*). The behaviour was characterized by complete quiescence interrupted at 1–1½ h intervals by one or more rapid rotary motions of the abdomen. Late in day 14 activity increased as indicated by the appearance of low-amplitude deflexions of the stylus between the major excursions (Fig. 1 *B*). Although the level of background activity gradually increased, this pattern continued until just prior to ecdysis.

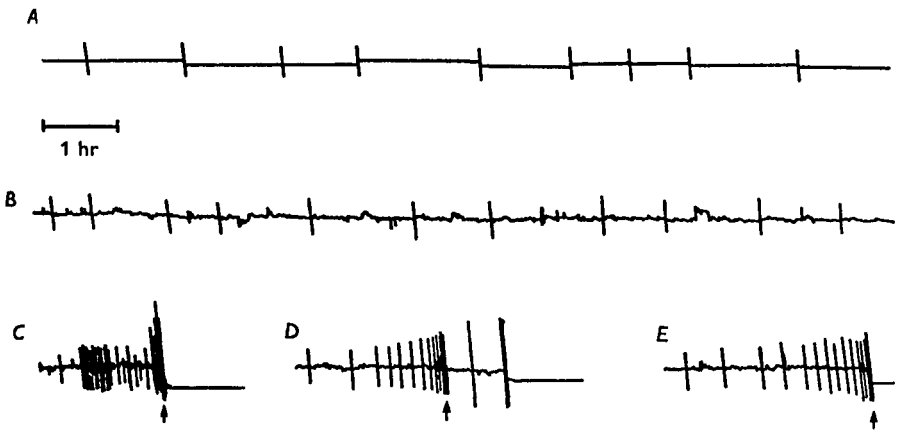

Fig. 1. Tracings of kymograph records showing the activity of developing and pharate silk moths. *A*, behaviour typical of *A. pernyi* pupae and animals up to and including the 13th day of adult development; *B*, behaviour typical of *A. pernyi* from the 15th day of development until shortly before adult eclosion. The pre-eclosion behaviour of: *C*, *H. cecropia*; *D*, *A. polyphemus* and *E*, *A. pernyi*. The arrows signal the moment of rupture of the pupal cuticle.

The eclosion of the moth was immediately preceded by a period of hyperactivity, the pre-eclosion behaviour. The pattern of this behaviour varied little between individuals but was species-specific (Truman & Riddiford, 1970). In the Cecropia silkmoth the pre-eclosion behaviour was the most stereotyped (Fig. 1 *C*). It began abruptly with a period of intense activity (about 0.5 h in duration) followed by an approximately equal period of reduced activity, and finally a short burst of hyperactivity accompanied by eclosion from the pupal exuviae. The average duration of this pre-eclosion pattern was 72 ± 9 min for 52 individuals.

In the two species of *Antheraea* (Fig. 1 *D*, *E*), the pre-eclosion behaviour was considerably less complex and less stereotyped than that of Cecropia. In both species the initiation of the hyperactive period was ill-defined. But once initiated, the activity gradually increased to a maximum during which time the pupal cuticle was split along the ecdysial lines. Pernyi moths then rapidly completed the process of

crawling from the old cuticle. Polyphemus, however, became quiescent after the initial rupturing of the cuticle. After a delay of 1–2 h these moths then completed the ecdysis.

2. The role of the pupal cuticle in the determination of the behaviour of the pharate moth

The behaviour of the moths prior to eclosion had little in common with their behaviour after eclosion. Indeed, pharate moths behaved essentially as pupae; their overt behaviour consisted primarily of pupal-like rotations of the abdomen. The simplest interpretation of this lack of 'adult behaviour' was that the confining

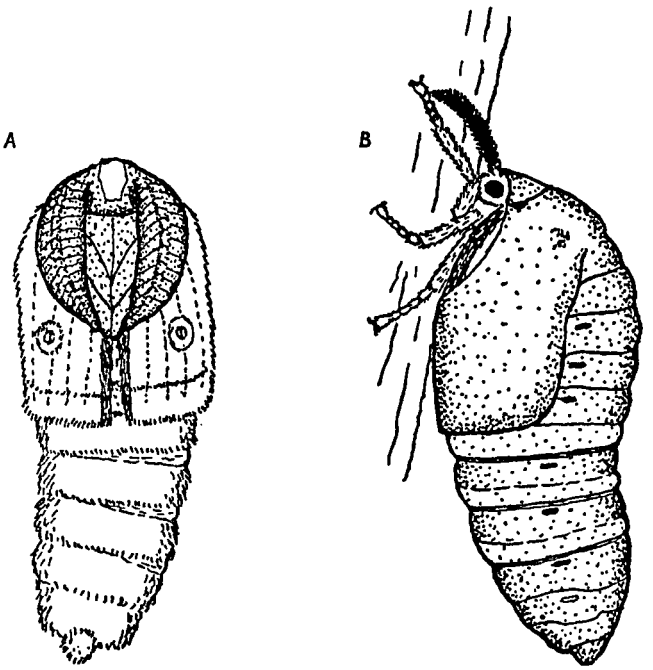

Fig. 2. Pharate *A. pernyi* moths: *A*, after removal of the entire pupal exuviae except for the facial shield; *B*, after removal of only the facial shield.

sheath of pupal cuticle restricted the behaviour of the animal. To test this possibility the pupal cuticle was removed from pharate Pernyi moths which had resorbed their moulting fluid and would not normally ecdyse for about 12 h.

After removal of the entire pupal cuticle with the exception of the small shield-shaped piece which covers the antennae, face and legs, the animals still showed the behaviour characteristic of a pupa (Fig. 2*A*). Movements were confined to pupal-like rotations of the abdomen.

The removal of only the facial shield yielded similar results. After its removal the moths thrashed out with their legs. But when such animals were placed on a vertical surface, they immediately clung to the surface and became quiescent (Fig. 2*B*). These animals displayed occasional rotary motions of the abdomen and made no attempt to shed the remainder of the exuviae until 10–13 h later – that is, until the normal ecdysis time.

As also noted by Lockshin (1971) the above behaviour can also be observed in

pharate moths from which the entire pupal exuviae has been peeled away. Prior to the time of their normal ecdysis gate these 'peeled' Pernyi moths made no attempt to spread their wings and their movements remained pupal.

3. *The 'eclosion' of 'peeled' moths*

As 'peeled' moths entered the eclosion gate typical for Pernyi, the frequency of abdominal movements gradually increased and culminated in an abrupt change in behaviour. Despite the complete removal of the old pupal cuticle hours earlier these moths displayed the entire behaviour that characterizes normal eclosion. During this 'ecdysis' one could distinguish three main types of movements: (1) a violent 'shrugging' of the wing bases; (2) a dorsal flexion of the animal accompanied by an extreme distension of the abdomen; and (3) rapid rotations of the abdomen. These three types of movement occurred a number of times in an apparently random sequence. This entire eclosion behaviour pattern lasted an average of 15 min.

During the later stages of 'eclosion' the labial glands began to secrete the cocoonase buffer which accumulated as a glistening drop on the face of the moth. Shortly thereafter the moths usually climbed upwards and eventually came to rest on a suitable substratum. They then initiated the slow rhythmic contractions of the abdomen which force blood into the wings to expand them (Judy & Gilbert, 1969). It is interesting to note that if newly emerged moths were confined in shell vials, they would struggle for 15–30 min, then try to spread their wings despite their unsuitable location.

4. *The influence of the central nervous system*

The above findings direct attention to a central control of the behaviour involved in emergence. The present experiments examined the role of the central nervous system in the first step of the sequence – the pre-eclosion behaviour. The stereotyped and complex pre-eclosion pattern of Cecropia proved ideal for this study.

The surgical procedures were confined to the head region. As summarized in Fig. 3, when all the major nerve trunks leading to the brain were severed the resulting moths still displayed the typical Cecropia pattern. Similarly, the excision of the subesophageal ganglion, an organ long implicated in the control of other motor functions in insects (Roeder, 1967), had no effect. By contrast, the excision of the brain drastically reduced the number of animals which displayed the characteristic behaviour.

The above results implied that a brain was necessary for the proper display of the pre-eclosion behaviour, but that the nervous connexions to the brain need not be intact. In eight Cecropia pupae the brain was completely isolated by its removal from the head and re-implantation into the abdomen. The resulting 'loose brain' moths consistently displayed the Cecropia pre-eclosion behaviour (Fig. 3).

These results permit two possible interpretations: either the brain itself is responsible for the Cecropia-specific behaviour pattern, or the brain serves to trigger the pattern which is programmed elsewhere in the nervous system. To differentiate between the two alternatives, a Pernyi brain was implanted into each of 11 brainless Cecropia pupae. All of the resulting moths displayed the typical Cecropia behaviour (Fig. 3), although they emerged during the typical Pernyi time (Truman & Riddiford,

1970). Thus it is clear that the brain serves only a triggering function. As we will see in the next section, the pre-eclosion behaviour is programmed in the abdominal ganglia.

Operation	Number	No. displaying behaviour	%
None	64	56	88
Optic nerves cut	4	4	100
Circumesophageal connectives cut	9	8	89
Subesophageal ganglion removed	24	21	88
Brain removed	16	2	12
Brain removed; cecropia brain implanted	8	8	100
Brain removed; pernyi brain implanted	11	10	91

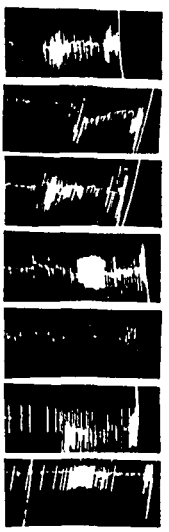

Fig. 3. The effects of surgery on the expression of the pre-eclosion behaviour of *H. cecropia*. The kymograph records show the typical behaviour in each experimental group.

Table 1. *The effects of homogenates of ganglia in stimulating eclosion of pharate* Antheraea pernyi *moths*

(Under the photoperiod conditions used in this experiment the normal eclosion gate occurs from 19.30 to 24.30. Pharate moths were injected at about 12.00.)

Tissue assayed	Number injected	Number eclosing within 3 h of injection	Number eclosing during following gate
Brain and corpora cardiaca of pharate adult Pernyi	30	30	0
Brain and corpora cardiaca of diapausing Pernyi pupa	7	0	7
Abdominal ganglia of pharate adult Pernyi	5	0	5

5. *The effect of the eclosion hormone*

(A) *On whole animals*

The eclosion of a pharate moth can be triggered by the injection of a homogenate of brain and corpora cardiaca prepared from another pharate animal (Truman & Riddiford, 1970). Injections of such homogenates were soon followed by the onset of the pre-eclosion behaviour and all animals ecdysed within 1½–3 h after treatment (Table 1). After eclosion these animals continued through the remaining portions of the emergence sequence. As seen in Table 1, homogenates prepared from the brains and corpora cardiaca of pupae or from the abdominal nerve cord of pharate adults

were inactive. Animals injected with these homogenates did not emerge until their normal eclosion gate 8–10 h later.

Treatment with active homogenates also stimulated the 'eclosion' of 'peeled' Pernyi. Two or three hours after injection the animals displayed the 'pantomime' eclosion behaviour followed by the secretion of the cocoonase buffer and the eventual spreading of the wings. The injection of the eclosion hormone can therefore completely replace the action of the brain in stimulating eclosion.

(B) *On isolated abdomens*

The abdomens of 23 pharate Cecropia were isolated as described under Methods. These were lightly anaesthetized with CO_2 and injected with a homogenate prepared from pharate adult brain and corpora cardiaca or with a placebo of 20 μl of Ringer. A thread was then waxed to the tip of the abdomen and the animals set

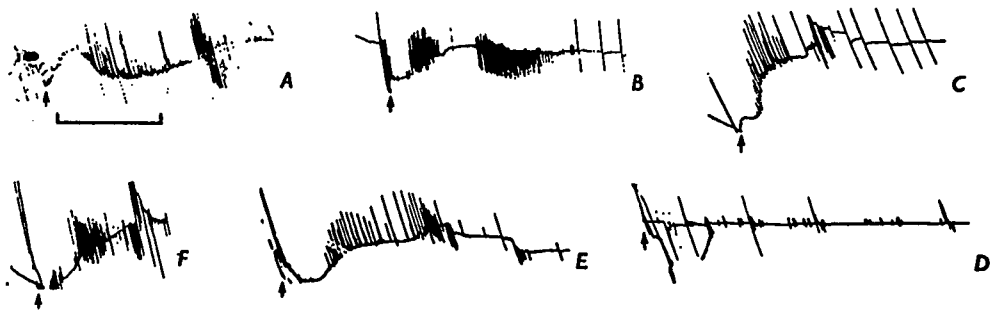

Fig. 4. Kymograph records showing the behaviour of isolated abdomens of *H. cecropia*. *A–E*, abdomens injected with homogenates containing eclosion hormone; *F*, an abdomen injected with Ringer's solution. The injections were given approximately 5 min before the animals were set up on the recording apparatus (arrow). The time mark equals 1 h.

up on kymographs. Of the 15 abdomens injected with the eclosion hormone, 10 displayed the typical three-part Cecropia pre-eclosion pattern (Fig. 4). About 20 min after injection the abdomens became hyperactive. This hyperactive period, which lasted 20–30 min, was then followed by a similar period of reduced activity and then by another period of hyperactivity. In some cases during this latter period the abdomen shed the pupal cuticle. Of the eight abdomens injected with Ringer none displayed the pre-eclosion behaviour (Fig. 4*F*). These results clearly show that the information necessary for the display of the pre-eclosion behaviour is programmed in the abdominal ganglia.

DISCUSSION

1. *The central control of the emergence sequence*

During the escape of the moth from the pupal exuviae and the cocoon one can identify five distinct behavioural patterns: the pre-eclosion behaviour, eclosion, the release of the cocoonase buffer, the post ecdysis activity, and the spreading of the wings. These patterns of behaviour are arranged in a specific temporal order and make up the 'emergence sequence'. The fact that even in a 'peeled' moth these behavioural elements still occur in the correct order clearly shows that the progression from one to the next does not require a stimulus–response relationship with the environment,

but rather must arise from an internal behavioural programme. Presumably the programming of this behaviour is analogous to the prepatterned organization of locust flight as shown by Wilson (1966). It should be noted that the control of the emergence sequence differs from the control of locust flight in one important respect. The latter is controlled by an oscillator which is programmed for a repetitive pattern of motor output. For eclosion the nervous system must contain the information for a non-repetitive sequence which lasts a number of hours and which involves a number of distinct behavioural elements.

From the behaviour of isolated abdomens which had been injected with the eclosion hormone it is obvious that the programme for the pre-eclosion behaviour and for the abdominal movements associated with eclosion is located in the abdominal ganglia. Also, it is of interest that after exposure to the eclosion hormone, the abdomen is capable of switching from one behavioural mode to the next without further influences from the head or thorax.

2. *The effect of environmental influences*

Although the switch from one part of the emergence sequence to the next is under some sort of internal control, the environment can influence the duration of certain parts of the sequence. In this respect the post-eclosion activity is the most easily influenced. In one moth this activity may consist of only a few steps, whereas another may crawl a number of metres before coming to a proper place and spreading its wings. But when no suitable site is provided, as when the newly emerged moth is placed in a shell vial, the animal will eventually attempt to spread its wings. Thus there is a period during which the environment can influence the post ecdysis activity. But if adverse conditions extend beyond this time, the moth must advance to the next part of the sequence.

The other parts of the emergence sequence seem to be influenced by the environment. The duration of the eclosion behaviour can be extended by 'peeling' the moth before the beginning of the emergence sequence. Also, Kafatos (1968) showed that the amount of cocoonase buffer released was greatly enhanced by restraining the newly emerged moth.

It is obvious then that the adult eclosion and associated behavioural events are controlled by a combination of peripheral and central influences. Fig. 5 summarizes the respective roles of these factors on the emergence sequence. Though the sequence of behaviour patterns arises from an internal programme which is apparently independent of the environment, this behavioural output can be quantitatively modified by sensory input. The benefit of this peripheral modulation of a central programme is self-evident. Without it the slightest environmental or physiological caprice might cause the moth to be trapped inside its exuviae or cocoon.

3. *The role of the eclosion hormone*

The role of a hormone in releasing a specific element of behaviour was shown in cockroaches about 10 years ago by Milburn & Roeder (1962). In that case the application of extract of corpora cardiaca to the nerve cord or its injection into the head capsule released a rhythmic bursting pattern in the phallic nerve of the male cockroach. The eclosion of silkmoths presents a similar case in that the injection of

the eclosion hormone precociously triggers the behaviour of the emergence sequence. Furthermore, the demonstration that eclosion hormone can be extracted from the blood of emerging moths (Truman, 1970) shows that this substance has a normal function in the behavioural physiology of the animal.

Prior to its emergence a moth exhibits little of the behaviour which characterizes it as an adult. Even when the pupal cuticle is removed, the pharate moth retains its pupal behaviour. In this respect eclosion effects a transition between the pupal and the adult behavioural modes. Thus it is seen that by stimulating eclosion, the eclosion hormone also serves to release the adult behaviour.

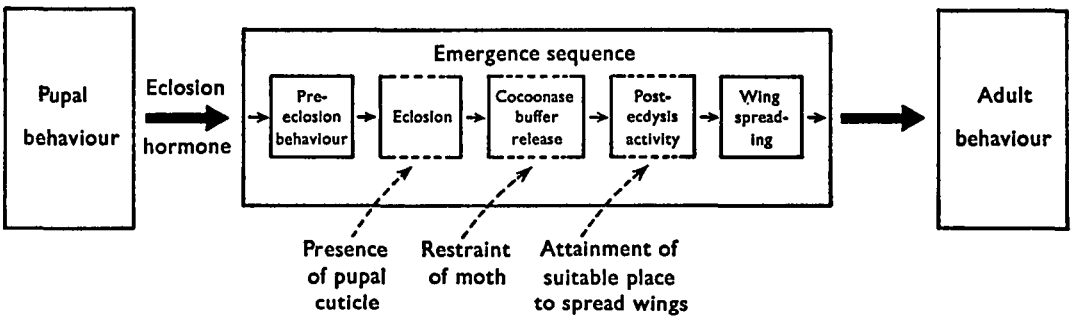

Fig. 5. A schematic representation of the control of the behaviour involved in the shedding of the pupal cuticle and the escape from the cocoon. The emergence sequence comprises a centrally programmed series of five elements of behaviour. The progression from one part to the next is effected by this central programme, but the duration of certain parts can be influenced by environmental factors. The activation of this programme is triggered by the eclosion hormone.

SUMMARY

1. In the giant silkmoths, adult eclosion is immediately preceded by a stereotyped series of abdominal movements – the pre-eclosion behaviour. The pattern of movements is species-specific and has a duration of about 1¼ h.

2. The pre-eclosion behaviour is followed sequentially by eclosion, the release of the labial gland secretion, the post-eclosion activity, and the spreading of the wings. These five elements of behaviour make up the emergence sequence.

3. The progression from one part of the behavioural sequence to the next is independent of stimuli provided by the pupal cuticle or cocoon, and therefore must be due to an internal programme. Thus, when the pupal cuticle was removed from pharate moths 12 h before their normal eclosion time, these 'peeled' animals continued to behave in a pupal fashion. But upon the arrival of the eclosion gate, the entire emergence sequence was displayed.

4. Through surgical manipulations the brain was shown to trigger the pre-eclosion behaviour. Moreover, this action of the brain was mediated by a neurosecretory hormone – the eclosion hormone.

5. Injections of extracts with eclosion-hormone activity triggered the precocious display of the emergence sequence by pharate moths.

6. When the eclosion hormone was injected into the isolated abdomens of pharate moths, these fragments performed the pre-eclosion behaviour and then shed the

surrounding piece of pupal cuticle. The information for the pre-eclosion behaviour and for the abdominal movements associated with eclosion must therefore be programmed in the abdominal ganglia. Moreover, after its 'activation' the abdomen can switch from one behaviour pattern to the next without influence from the higher centres.

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