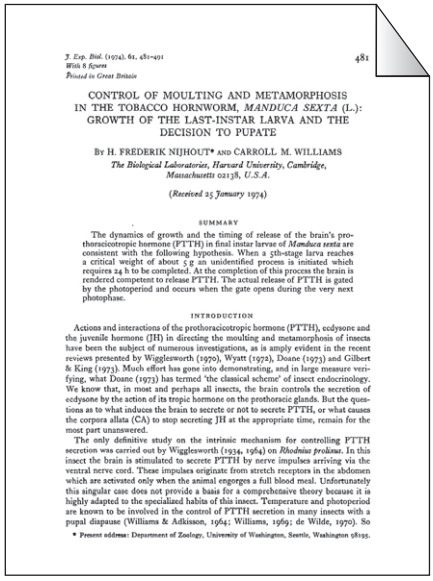
JEB Classics is an occasional column, featuring historic publications from *The Journal of Experimental Biology*. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work. A PDF of the original paper is available from the JEB Archive (http://jeb.biologists.org/).

JEB Classics

WHEN IS WEIGHT CRITICAL?



J. Exp. Biol. (1974), 61, 481–491
With 8 figures
Printed in Great Britain

481

CONTROL OF MOULTING AND METAMORPHOSIS IN THE TOBACCO HORNWORM, *MANDUCA SEXTA* (L.): GROWTH OF THE LAST-INSTAR LARVA AND THE DECISION TO PUPATE

BY H. FREDERIK NIJHOUT* AND CARROLL M. WILLIAMS
The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138, U.S.A.

(*Received 25 January 1974*)

SUMMARY

The dynamics of growth and the timing of release of the brain's prothoracicotropic hormone (PTTH) in final instar larvae of *Manduca sexta* are consistent with the following hypothesis. When a 5th-stage larva reaches a critical weight of about 5 g an unidentified process is initiated which requires 24 h to be completed. At the completion of this process the brain is rendered competent to release PTTH. The actual release of PTTH is gated by the photoperiod and occurs when the gate opens during the very next photophase.

INTRODUCTION

Actions and interactions of the prothoracicotropic hormone (PTTH), ecdysone and the juvenile hormone (JH) in directing the moulting and metamorphosis of insects have been the subject of numerous investigations, as is amply evident in the recent reviews presented by Wigglesworth (1970), Wyatt (1972), Doane (1973) and Gilbert & King (1973). Much effort has gone into demonstrating, and in large measure verifying, what Doane (1973) has termed 'the classical scheme' of insect endocrinology. We know that, in most and perhaps all insects, the brain controls the secretion of ecdysone by the action of its tropic hormone on the prothoracic glands. But the questions as to what induces the brain to secrete or not to secrete PTTH, or what causes the corpora allata (CA) to stop secreting JH at the appropriate time, remain for the most part unanswered.

The only definitive study on the intrinsic mechanism for controlling PTTH secretion was carried out by Wigglesworth (1934, 1964) on *Rhodnius prolixus*. In this insect the brain is stimulated to secrete PTTH by nerve impulses arriving via the ventral nerve cord. These impulses originate from stretch receptors in the abdomen which are activated only when the animal engorges a full blood meal. Unfortunately this singular case does not provide a basis for a comprehensive theory because it is highly adapted to the specialized habits of this insect. Temperature and photoperiod are known to be involved in the control of PTTH secretion in many insects with a pupal diapause (Williams & Adkisson, 1964; Williams, 1969; de Wilde, 1970). So

* Present address: Department of Zoology, University of Washington, Seattle, Washington 98195.

Lynn Riddiford discusses Frederik Nijhout and Carroll Williams' 1974 paper entitled 'Control of moulting and metamorphosis in the tobacco hornworm, *Manduca sexta* (L.): growth of the last-instar larva and the decision to pupate'.

A copy of the paper can be obtained from http://jeb.biologists.org/cgi/content/abstract/61/2/481

In insects, all growth occurs before metamorphosis so that the final size of the larva determines the size of the adult. This size is species-specific but how is this final larval size determined? In this classic paper, Nijhout and Williams (Nijhout and Williams, 1974a) used the tobacco hornworm *Manduca sexta* to develop the concept of a critical weight necessary for metamorphosis. They knew that the onset of metamorphosis, as signaled by the cessation of feeding and the voiding of the gut contents (the gut purge), was initiated by the molting hormone ecdysone from the prothoracic glands upon stimulation by the prothoracicotropic hormone (PTTH) from the brain (Truman and Riddiford, 1974). In *M. sexta* reared in a defined photoperiod and temperature regime, this PTTH release can occur only during a particular time period each day (known as the 'gate') and the gut purge occurs 12–15 h later. When Nijhout and Williams fed larvae on a series of diets with progressively less nutrients, the growth rate was slowed, the gut purge was delayed and the final weight of the larvae was progressively less. But when the growth curves of the larvae from the various groups were aligned at the time of PTTH release, they found that the curves intersected at approximately 24 h before the gate for PTTH release and at a weight of approximately 5 g. This weight was considered the 'critical weight' at which the larvae became committed to release PTTH. Further experiments showed that if the larvae above this critical weight were starved, they would release PTTH on time; but if starved below this weight, PTTH release was delayed. They then hypothesized that a process was initiated at the critical weight that requires approximately 24 h to be completed for the brain to be competent to release PTTH.

In their subsequent paper, Nijhout and Williams (Nijhout and Williams, 1974b) showed that a steep decline of juvenile hormone (JH) in the hemolymph begins at approximately 5 g and reaches undetectable levels approximately 24 h later and that this decline is critical for PTTH release. Application of exogenous JH prevented that release. Moreover, removal of the corpora allata that produce JH from larvae early in the final instar allowed these larvae to release PTTH for the gut purge earlier. Nijhout and Williams hypothesized that the corpora allata were turned off at the time when the larva attained the critical weight, and the subsequent decline of JH rendered the brain competent to release PTTH. Later studies on *M. sexta* confirmed that JH drops to undetectable levels at this time, coincident with a steep rise in JH esterase activity (Baker et al., 1987; Browder et al., 2001). Also, JH was found to inhibit both the release of PTTH by the brain and the acquisition of competence of the prothoracic glands to respond to PTTH (Rountree and Bollenbacher, 1986; Watson and Bollenbacher, 1988).

In the past 20 years, the search for the physiological mechanisms underlying size control and critical weight has been extended to other insects. In particular, recent studies in the silkworm *Bombyx mori* have called into question the role of JH in the inhibition of PTTH release. In *B. mori*, the JH titer in the final instar declines similarly to that of *M. sexta* (Niimi and Sakurai, 1997). PTTH is low but detectable in the hemolymph during the early part of the instar, and then shows a sharp transient rise that coincides with the onset of wandering behavior (Mizoguchi et al., 2001). JH can stimulate a small increase in the PTTH titer early in the instar but does not appear to suppress PTTH release when given the day before the onset of wandering (Mizoguchi, 2001). Similar studies that directly measure PTTH levels have not been done in *M. sexta*. JH, however, does have a direct effect on the steroidogenic activity of the prothoracic glands in *B. mori*. Studies on isolated glands *in vitro* show that JH suppresses the transcription of *spook* (Yamanaka et al., 2007), which encodes a key enzyme for one of the early

steps of ecdysone biosynthesis (Ono et al., 2006). In both *B. mori* and *M. sexta* prothoracic glands, the *spook* transcript increases sharply in the mid-feeding phase of the final instar (Ono et al., 2006), coincident with the decline of JH. In *M. sexta* glands isolated on the day before gut purge, PTTH caused a rapid increase in Spook protein and its phosphorylation whereas it had little effect on the other enzymes in the ecdysone biosynthetic pathway (Rewitz et al., 2009). Clearly the disappearance of JH after the attainment of critical weight allows increased ecdysone synthesis by the prothoracic glands in response to PTTH, but how it leads to the pulse of PTTH that initiates metamorphosis is still a mystery.

Since the identification of the critical weight as the key factor in determining insect size, the question has been how this size is assessed (Nijhout, 2003). Recent studies in *Drosophila melanogaster* show that the size of the prothoracic glands serves as an indicator of body size and thus when critical weight has been attained. When these glands were stimulated to grow larger by increasing insulin signaling within the gland, the larvae pupariated at lower than normal weights; when growth of the glands was retarded by preventing insulin signaling, the larvae fed longer and formed abnormally large puparia (Caldwell et al., 2005; Colombani et al., 2005; Mirth et al., 2005). The effect on body size was specific to insulin signaling-induced growth of the gland, which was accompanied by upregulation of enzymes in the ecdysone biosynthetic pathway (Colombani et al., 2005). The increased size of the prothoracic gland at critical weight leads to an increased basal level of ecdysteroid that initiates the early events of metamorphosis, such as expression of the ecdysone-induced E74 transcription factor (Caldwell et al., 2005; Colombani et al., 2005) and the onset of ecdysone-regulated patterning of the imaginal discs (Mirth et al., 2009).

In *M. sexta*, the final size of the larva is dependent only on the quality of the diet consumed after reaching critical weight because the timing of PTTH release is fixed by the endocrine events described above that were set in motion at critical weight. Thus, those feeding on suboptimal diets form smaller pupae. By contrast, in *D. melanogaster*, the terminal growth period after critical weight is more plastic as it decreases when the larvae are starved (Mirth et al., 2005; Stieper et al., 2008) but increases when the larvae have been fed on a suboptimal diet (Layalle et al., 2008). Here again the prothoracic gland appears to play a key role. The level of Target of Rapamycin (TOR) signaling, which coordinates cell growth with nutrient (amino acids) input (Hietakangas and Cohen, 2009), in the prothoracic gland at the time of critical weight was found to determine the timing of the later ecdysone pulse that initiates the cessation of feeding and the onset of wandering (Layalle et al., 2008). By contrast, the level of TOR signaling in the PTTH neurons had no effect. The underlying mechanism is not yet known, although both increased levels of ecdysone biosynthetic enzymes and increased sensitivity to PTTH are thought to be involved (Layalle et al., 2008).

Interestingly, *D. melanogaster* larvae that have their corpora allata removed (allatectomized), and thus lack JH, form smaller than normal puparia but will attain the normal size when fed JH-containing diet during the final larval instar (Riddiford et al., 2010). Neither critical weight nor the terminal growth period is altered in these allatectomized larvae (A. W. Shingleton and C. K. Mirth, personal communication). Instead, their decreased size can be accounted for by their reduced growth rate. The physiological mechanisms underlying this effect of JH on growth rate are currently under study.

The role of critical weight in the evolution of body size has also been studied recently. Over the 30 years of laboratory rearing since the initial studies of Nijhout and Williams, *M. sexta* larvae in this colony underwent a 50% increase in body size. This increase in size was found to be due to a combination of changes in critical weight, growth rate and duration of the terminal growth period (D'Amico et al., 2001). Subsequent selection experiments on both body size and development time showed that an increase in body size was primarily due to increases in the terminal growth period and the growth rate whereas decreased body size was dependent on decreases in critical weight and growth rate, apparently due to conflicting effects of simultaneous directional selection on these underlying processes (Nijhout et al., 2010). Whether this model holds for other insects such as *D. melanogaster*, where the relationship between critical weight and final body size is more complex, requires further study.

Clearly, critical weight is an important but cryptic milestone for an insect larva in determining its final size. Crossing the critical weight threshold initiates a suite of physiological mechanisms that determine the duration of feeding thereafter and thus dictate the size of the adult. Details of these processes, however, vary amongst insects. Obviously, critical weight has been a fertile concept for the past 37 years, and it will continue to guide us in elucidating how insects adapt to the nutritional constraints of their different ecological niches.

10.1242/jeb.049098

Lynn M. Riddiford
Janelia Farm Research Campus,
Howard Hughes Medical Institute
riddifordl@janelia.hhmi.org

References

Baker, F. C., Tsai, L. W., Reuter, C. C. and Schooley, D. A. (1987). *In vivo* fluctuation of JH, JH acid, and ecdysteroid titer, and JH esterase activity, during development of fifth stadium *Manduca sexta*. *Insect Biochem.* **17**, 989-996.
Browder, M. H., D'Amico, L. J. and Nijhout, H. F. (2001). The role of low levels of juvenile hormone esterase in the metamorphosis of *Manduca sexta*. *J. Insect Sci.* **1**, 1-4.
Caldwell, P. E., Walkiewicz, M. and Stern, M. (2005). Ras activity in the *Drosophila* prothoracic gland regulates body size and developmental rate via ecdysone release. *Curr. Biol.* **15**, 1785-1795.
Colombani, J., Bianchini, L., Layalle, S., Pondeville, E., Dauphin-Villemant, C., Antoniewski, C., Carré, C., Noselli, S. and Léopold, P. (2005). Antagonistic actions of ecdysone and insulins determine final size in *Drosophila*. *Science* **310**, 667-670.
D'Amico, L. J., Davidowitz, G. and Nijhout, H. F. (2001). The developmental and physiological basis of body size evolution in an insect. *Proc. R. Soc. Lond. B* **268**, 1589-1593.
Hietakangas, V. and Cohen, S. M. (2009). Regulation of tissue growth through nutrient sensing. *Annu. Rev. Genet.* **43**, 389-410.
Layalle, S., Arquier, N. and Leopold, P. (2008). The TOR pathway couples nutrition and developmental timing in *Drosophila*. *Dev. Cell* **15**, 568-577.
Mirth, C., Truman, J. W. and Riddiford, L. M. (2005). The role of the prothoracic gland in determining critical weight for metamorphosis in *Drosophila melanogaster*. *Curr. Biol.* **15**, 1796-1807.
Mirth, C. K., Truman, J. W. and Riddiford, L. M. (2009). The Ecdysone receptor controls the post-critical weight switch to nutrition-independent differentiation in *Drosophila* wing imaginal discs. *Development* **136**, 2345-2353.
Mizoguchi, A. (2001). Effects of juvenile hormone on the secretion of prothoracicotropic hormone in the last- and penultimate-instar larvae of the silkworm, *Bombyx mori*. *J. Insect Physiol.* **47**, 767-775.
Mizoguchi, A., Ohashi, Y., Hosoda, K., Ishibashi, J. and Kataoka, H. (2001). Developmental profile of the changes in prothoracicotropic hormone titer in hemolymph of the silkworm *Bombyx mori*: correlation with ecdysteroid secretion. *Insect Biochem. Mol. Biol.* **31**, 349-358.
Niimi, S. and Sakurai, S. (1997). Development changes in juvenile hormone and juvenile hormone acid titers in the hemolymph and *in vitro* juvenile hormone synthesis by corpora allata of the silkworm, *Bombyx mori*. *J. Insect Physiol.* **43**, 875-884.
Nijhout, H. F. (2003). The control of body size in insects. *Dev. Biol.* **261**, 1-9.
Nijhout, H. F. and Williams, C. M. (1974a). Control of moulting and metamorphosis in the tobacco hornworm, *Manduca sexta* (L.): growth of the last-instar larva and the decision to pupate. *J. Exp. Biol.* **61**, 481-491.
Nijhout, H. F. and Williams, C. M. (1974b). Control of moulting and metamorphosis in the tobacco hornworm, *Manduca sexta* (L.): cessation of juvenile hormone secretion as a trigger for pupation. *J. Exp. Biol.* **61**, 493-501.
Nijhout, H. F., Roff, D. A. and Davidowitz, G. (2010). Conflicting processes in the evolution of body size and development time. *Philos. Trans. R. Soc. Lond. B* **365**, 567-575.
Ono, H., Rewitz, K. F., Shinoda, T., Itoyama, K., Petryk, A., Rycbczynski, R., Jarcho, M., Warren, J. T., Marqués, G., Shimell, M. J. et al. (2006). *Spook* and *Spookier* code for stage-specific components of the ecdysone biosynthetic pathway in Diptera. *Dev. Biol.* **298**, 555-570.

Rewitz, K. F., Larsen, M. R., Lobner-Olesen, A., Rybczynski, R., O'Connor, M. B. and Gilbert, L. I. (2009). A phosphoproteomics approach to elucidate neuropeptide signal transduction controlling insect metamorphosis. *Insect Biochem. Mol. Biol.* **39**, 475-483.
Riddiford, L. M., Truman, J. W., Mirth, C. K. and Shen, Y. (2010). A role for juvenile hormone in the prepupal development of *Drosophila melanogaster*. *Development* **137**, 1117-1126.
Rountree, D. B. and Bollenbacher, W. E. (1986). The release of the prothoracicotropic hormone in the tobacco hornworm, *Manduca sexta*, is controlled intrinsically by juvenile hormone. *J. Exp. Biol.* **120**, 41-58.
Stieper, B. C., Kupershtok, M., Driscoll, M. V. and Shingleton, A. W. (2008). Imaginal discs regulate developmental timing in *Drosophila melanogaster*. *Dev. Biol.* **321**, 18-26.
Truman, J. W. and Riddiford, L. M. (1974). Physiology of insect rhythms. III. The temporal organization of the endocrine events underlying pupation of the tobacco hornworm. *J. Exp. Biol.* **60**, 371-382.
Watson, R. D. and Bollenbacher, W. E. (1988). Juvenile hormone regulates the steroidogenic competence of *Manduca sexta* prothoracic glands. *Mol. Cell. Endocrin.* **57**, 251-259.
Yamanaka, N., Honda, N., Osato, N., Niwa, R., Mizoguchi, A. and Kataoka, H. (2007). Differential regulation of ecdysteroidogenic P450 gene expression in the silkworm, *Bombyx mori*. *Biosci. Biotechnol. Biochem.* **71**, 2808-2814.