times on a period of 15 min (a measurement every 5 min). All measurements of intraocular pressure were performed between 13.00 and 15.00. Statistical differences were calculated with the paired t-test.

Results. Addicted patients showed a lower basal value of intraocular pressure in both eyes as compared to control subjects (table). The instillation of naloxone in the right eye of addicted patients resulted in a significant increase ($p < 0.01$, as compared to the control left eye) in intraocular pressure, to a level similar to that of saline-treated eyes of non addicted healthy volunteers ($p > 0.05$). No difference in intraocular pressure of right and left eye was apparent after naloxone instillation in control subjects.

Discussion. The present results confirm our previous observation that patients addicted to morphine show an increased aqueous outflow associated with a decrease in intraocular pressure[5]. Moreover, since instillation of naloxone caused a remarkable increase in intraocular pressure in addicted patients, it is possible to speculate that local opiate receptors are involved in the regulation of intraocular pressure in man. Intraocular injection of morphine reduces intraocular pressure in rabbit[5]. Opiate receptors have been described in the iris of rabbit[1] and man[3]. Furthermore, the existence of opioid peptides has recently been demonstrated in the ox eye[6]. Thus, it is possible that the same population of opiate receptors in the eye affect pupillary diameter and intraocular pressure. Against this possibility is an old observation that morphine may lower intraocular pressure without increasing miosis[7].

Instillation of naloxone induces mydriasis in addicted patients[4]. This effect may be mediated by the liberation of noradrenaline accumulated during morphine abuse. In fact, morphine inhibits noradrenaline release in the cat nictitating membrane[8] and in the mouse vas deferens[9]. The same mechanism may be postulated in the effect of naloxone on intraocular pressure. Thus, naloxone applied topically to the eye provokes a local miniature withdrawl reaction that is limited to the functions of pupil and intraocular pressure, without general effect. In conclusion, beside the pupillary test described by Fanciullacci et al.[4], the naloxone test on intraocular pressure seems to be a useful screening method for detecting morphine addiction.

Effect of ocular instillation of naloxone on the intraocular pressure of morphine-addicted (n = 10) and control (n = 4) subjects. The drug was applied to the right eye 1 h after the basal measurements, while only saline was instilled in the left eye. Values are mean ± SE (in mmHg)

	Times of observation (after naloxone instillation)				
	Basal	30 min	35 min	40 min	45 min
Addicted					
Right eye	10.1 ± 0.8**	14.4 ± 0.8ff	14.7 ± 0.5ff	14.8 ± 0.3ff	14.0 ± 0.6ff
Left eye	10.0 ± 0.4**	10.1 ± 0.2	10.2 ± 0.2	9.8 ± 0.3	9.7 ± 0.4
Controls					
Right eye	14.0 ± 0.4	14.6 ± 0.4	13.8 ± 0.6	13.8 ± 0.6	13.7 ± 0.7
Left eye	14.6 ± 0.5	14.5 ± 0.7	13.5 ± 0.6	13.6 ± 0.5	13.4 ± 0.4

** Significantly different as compared to correspondent eye in control subjects ($p < 0.01$, paired t-test); ff Significantly different as compared to saline-treated left eye ($p < 0.01$, paired t-test).

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Photoperiodically induced delayed insect metamorphosis: a larval oligopause in *Diatraea saccharalis*

R.M. Roe[1], A.M. Hammond, Jr[2], and B.J.R. Philogène

Department of Entomology, Louisiana Agricultural Experiment Station, Center for Agricultural Sciences and Rural Development, Louisiana State University, Baton Rouge (Louisiana 70803, USA), and Department of Biology, University of Ottawa, Ottawa (Canada KIN 6N5), 22 November 1983

Summary. Sugarcane borers enter a state of delayed metamorphosis when exposed to a 12-h photophase at 21 °C. Larval feeding, growth, and molting continues but pupation is suppressed under these conditions.
Key words. Diapause; oligopause; sugarcane borer; *Diatraea saccharalis;* Lepidoptera; Pyralidae.

Diapause has long been recognized as a state of suppressed growth and development[3-6]. Delayed metamorphosis in the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Pyralidae)[7,8], was thought to be similar to the closely related and well studied diapause of the southwestern cornborner, *D. grandiosella* Dyar, where there is a state of delayed pupation and no feeding or growth[9,10]. Sugarcane borer larvae, however, in response to a 12-h photophase at 21 °C enter a state of delayed metamorphosis, where pupation is suppressed but larval feeding, growth, and moulting continues. Mansingh referred to this condition as oligopause[5] but had no definitive studies as proof. Delayed metamorphosis in the sugarcane borer was photoperiodically induced for both male and female borers within the first 2 stadia. Delayed metamorphosis in borers reared[11] from egg hatch at 21 °C on photophases of fixed duration from 10–13 h was greater than 90% (fig. 1a) with shorter and longer photophases producing less than 60% induction (a type 1 response curve[3]). The percent delayed pupation was greatly reduced when borers were transferred from LD 14:10 to LD 12:12 at 10 days or later, but approximately 100% if the trans-

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fer was made at the 5th day or before (fig. 1b). It was also found that delayed metamorphosis in borers reared at LD 12:12 for the first 15 days could not be totally reversed by a subsequent shift to LD 14:10.

Delayed development although inducible within the first 2 stadia, was not expressed until what normally would be the last stadium under LD 14:10 conditions. The completion of each stadium for female borers reared at LD 14:10 and LD 12:12, 21 °C was identical ($\alpha = 0.05$, t-test) for the first 5 stadia (fig. 2). The duration of the 6th stadium, however, was 10 days longer at LD 12:12. The female mean head capsule width was also identical ($\alpha = 0.05$, t-test) for the first 5 stadia for borers at LD 14:10 and LD 12:12, 21 °C (fig. 3a). The growth ratio[11], the ratio of the next-to-last head capsule width divided by that of the first, at LD 12:12 was 1.5 times that at LD 14:10. The ratio was 7.71 at LD 12:12 and 4.70 at LD 14:10. Since there was no significant difference in head capsule width between LD 14:10 and LD 12:12 from the 1st through the 5th stadium and there was a prolonged 6th stadium at LD 12:12, the increased growth (or growth ratio) at LD 12:12 appeared to be initiatd in the 6th stadium or what would be the last stadium under LD 14:10 conditions. Furthermore, in the 6th stadium (at 40 days, fig. 3b) LD 14:10 and LD 12:12 borers were equal in weight ($\alpha = 0.05$, t-test). During the next 3 days, however, LD 14:10 borers lost weight and pupated while LD 12:12 borers continued to feed and grow. Between the time of induction early in development and the initiation of delayed metamorphosis in the 6th stadium, LD 14:10 and LD 12:12 borers moulted on the same time schedule (fig. 2) and grew at approximately the same rate (fig. 3). Sugarcane borers during this period, at LD 12:12 did not accumulate lipid as was the case for the southwestern cornborer in preparation for diapause[12]. The sugarcane borer also has a sexual, developmental polymorphism[11] but the relative effects of LD 14:10 and LD 12:12 on development was identical for both sexes and between instar groups.

Once the initiation of delayed metamorphosis occurred in the sixth stadium, the sugarcane borer at LD 12:12 continued to feed, gain weight (fig. 3b) and molt (fig. 2) while 6th stadium LD 14:10 borers pupated. The rate of growth of LD 12:12 borers was relatively unchanged through the time of pupation of LD 14:10 borers and until the induction of pupation at 82 days (fig. 3). Induction of pupation after 82 days resulted in high borer mortality. The maximum larval and 1-day-old pupal wet weight at LD 12:12 was twice that at LD 14:10. There was a significant increase with age ($\alpha = 0.05$, tuckey's ω-procedure) in wet weight, dry weight, and percent water but

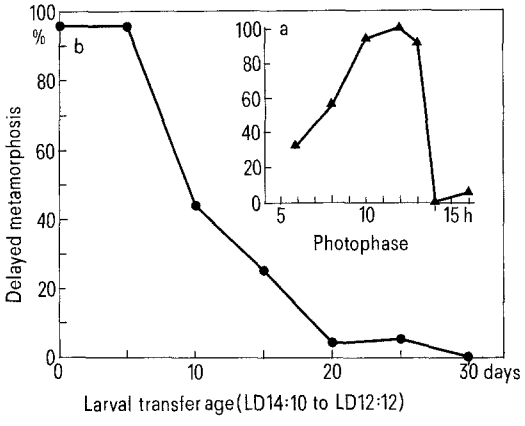

Figure 1. Photoperiodic induction of delayed metamorphosis early in larval development. Borers were reared at 21 °C on an artificial diet[11]. The criterion for delayed metamorphosis was larval stage duration exceeding the mean +2 SD of the larval stage duration for borers reared from egg hatch at LD 14:10, 21 °C. *a* The relationship between a fixed photophase duration and the induction of delayed metamorphosis in larvae reared from egg hatch on a 24-h photoperiod. Each percentage was derived from at least 73 borers. *b* The relationship of the cumulative larval age of transfer from LD 14:10 to LD 12:12 to the induction of delayed metamorphosis. Each percentage was derived from at least 25 borers.

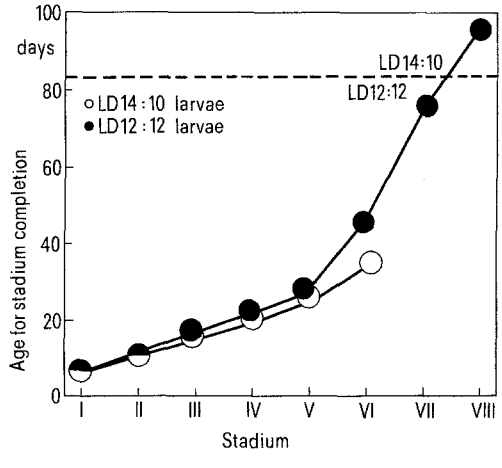

Figure 2. Larval cumulative age from egg hatch for the completion of each stadium for female borers reared at LD 14:10 or LD 12:12, 21 °C. Larvae were transferred from LD 12:12 to LD 14:10 at 82 days to induce pupation. The standard error of the mean did not exceed the size of the symbols. The total number of borers examined was 52.

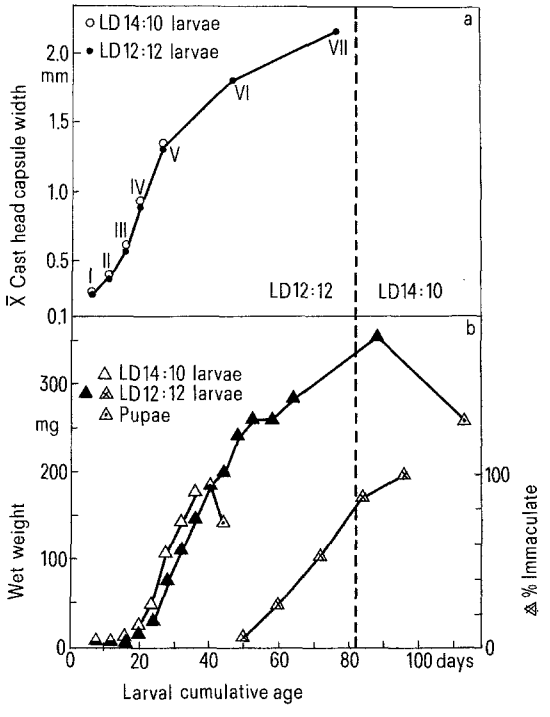

Figure 3. Head capsule and wet weight growth for female borers reared from egg hatch at either LD 14:10 or LD 12:12, 21 °C. Borers were transferred from LD 12:12 to LD 14:10 at 82 days to induce pupation. The standard error of the mean did not exceed the size of the symbols. *a* The cast head capsule width was mesured for the first through the next-to-last stadium. The last stadium, cast head capsule was split along the epicranial suture and was not measured. The total number of borers examined was 52. *b* Each wet weight represents the mean of at least 11 insects. The cumulative percentage of immaculate borers[8] appearing at LD 12:12 was derived from at least 16 borers.

not in percent lipid (unchanged) for undisturbed LD 12:12 larvae at 40, 84, and 109 days. Immaculate larvae[8] (fig. 3b) appeared at LD 12:12 when larvae moulted from the 6th to the 7th or the 7th to the 8th stadium. Immaculate sugarcane borer larvae (borers without pinacular pigmentation) always gained weight and were able to moult. In contrast, the southwestern corn borers did not feed or gain weight during diapause or as immaculate larvae (personal communication, G.M. Chippendale).

Burges[14] found that larval Khapra beetles, *Trogoderma granarium* Everts, occasionally fed, gained weight, and molted during delayed pupation, but it was not clear whether this was quiescence (cold or heat stupor) or diapause because in some cases the delay in pupation could be terminated with fresh food[13,14]. A number of other isolated field observations with various insect species were equally as puzzling[5,15,16]. Mansingh proposed an alternative physiological strategy to quiescence and diapause that allowed insects to deal with seasonal changes of a short-term and moderate nature[5]. He referred to this condition as oligopause. Unfortunately, no definitive studies were available as proof. It appears in the sugarcane borer from both field observations[17] and laboratory study (figs 1–3) that feeding and growth is possible during photoperiodically induced delayed metamorphosis. Delayed metamorphosis in the sugarcane borer prevents adult reproduction during a generally unfavorable winter season; but the option of feeding allows the borer to also take advantage of any intermittent periods when favorable conditions for growth may exist. Feeding during delayed metamorphosis is more than simply for the maintenance of a minimum threshold size needed to successfully complete metamorphosis and adult reproduction since borers are able to double their normal weight (fig. 3b). A greater weight increases the chance of surviving periods of unfavorable conditions when feeding is impossible and may affect fecundity in the spring. The example of delayed metamorphosis in the sugarcane borer is an alternative physiological strategy to the well known conditions of quiescence and diapause. This condition in the sugarcane borer is classified by Mansingh[5] as oligopause but is classified by others as weak diapause[3].

1 Present address: Department of Entomology, Box 7613, North Carolina State University, Raleigh, North Carolina 27695-7613, USA.
2 Reprint requests to A.M.H. Jr, Department of Entomology, Louisiana State University, Baton Rouge, Louisiana 70803, USA.

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Mast cells are present during angiogenesis in the chick extraembryonic vascular system

D.J. Wilson

Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, Cleveland Street, London W1P6DB (England), 17 October 1983

Summary. The extraembryonic vascular membranes of 3-day–18-day chick embryos were examined for the presence of mast cells. As early as 3.5 days mast cells were found on the area vasculosa. It is suggested that these cells have a role in angiogenesis of the chick extraembryonic vascular system.

The extraembryonic membranes of the chick embryo have been frequently used to assay potential angiogenic or anti-angiogenic agents. Most often the mature, non-growing vessels of the chorio-allantoic membrane (CAM)[2] have been employed to demonstrate the capacity of various agents such as infarcted myocardial tissue[3], lymphocytes and embryonic tissues[4], to induce new blood vessel growth. In particular, this system has provided a useful assay for determining the angiogenic properties of neoplastic tissues[5].

The immature, growing blood vessels of the yolk sac vasculature (area vasculosa) of early chick embryos have recently been used to show that embryonic angiogenesis can be promoted and inhibited by applying specific angiogenic and anti-angiogenic molecules[6]. The experiments demonstrated that locally applied protamine (a basic protein) inhibited the growth and expansion of the blood vessels over the yolk sac, causing the formation of a large avascular zone; and that this inhibitory property of protamine could be overcome by heparin. The discovery of the angiogenic properties of heparin and the inhibitory properties of protamine resulted from a series of experiments in which mast cells, and particularly mast cell heparin, were implicated. For example, mast cell accumulation was found to precede the ingrowth of new capillary sprouts at a tumor site[7]; heparin released by mast cells stimulated migration of capillary endothelial cells in vitro[8]; heparin enhanced tumor angiogenesis on the chick CAM[6], and protamine (an antagonist of heparin) blocked mast cell or heparin stimulation of capillary endothelial cell migration in vitro[8].

In view of these findings it is possible that mast cells, and the heparin they produce, have a role in normal embryonic angiogenesis. If the angiogenesis accompanying embryonic development is promoted by mast cell heparin, then it is important to demonstrate the presence of mast cells in regions of vascular development. Thus extraembryonic membranes of chick em-