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Hormonal control of pheromone responsiveness in the male black cutworm *Agrotis ipsilon*

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Abstract. In Lepidoptera, reproduction is linked to chemical communication between conspecific partners. When exposed to the female sex pheromone, males respond by exhibiting typical sexual behaviour which leads to mating. Here we show that presence of the juvenile hormone producing gland (corpora allata) of the male black cutworm, *Agrotis ipsilon*, is necessary for pheromone responsiveness. Allatectomized males do not show any sexual behaviour, although their antennal olfactory system is functional. Allatectomized males implanted with active corpora allata recover full pheromone receptivity. It is suggested that reproductive processes are synchronized in males and females through endocrine control; timing of the mating activity could serve as an adaptive strategy linked to the migratory behaviour of this species.

Key words. Lepidoptera; Noctuidae; *Agrotis ipsilon*; black cutworm; juvenile hormone; allatectomy; pheromone reception; sexual behaviour; fenoxycarb; KK-42.

In insects, reproduction is dependent on optimum synchronization of physiological and behavioural maturation processes in both males and females. In most Lepidoptera, males respond to a sex pheromone produced by a gland in the abdominal tip of the conspecific females. Reception of the pheromonal blend by male antennae leads to sexual behaviour and mating. Recent studies have shown that juvenile hormone (JH), produced in the corpora allata (CA), regulates the female sexual behaviour (pheromone production and release) of a noctuid moth, *Pseudaletia unipuncta*[1].

For most moth species, pheromone olfactory receptors become functional soon after emergence, and mating occurs early in adult life. However in some moth families, e.g. Noctuidae, a delay occurs between emergence and maximum responsiveness to sex pheromone, indicating some possible endogenous maturation process[2,3]. Moreover, the noctuid moth, *Agrotis ipsilon*, is less likely to be caught in sex-attractant baited traps during its autumn southward flight than on its way northwards. The seasonal decrease in response to sex attractants has been associated with an adult diapause linked to unfavorable climatic conditions[4-6]. This adaptive strategy of Lepidoptera is often characterized by an arrest of oocyte development in females[7], but characterization of the male adult diapause remains unclear.

Although the role of endocrine factors in the control of male sexual behaviour has been described in Orthoptera[8], their role in Lepidoptera is almost undocumented. However, in the autumnal adult diapause of the monarch butterfly, *Danaus plexippus*, the male reproductive glands (tubular and accessory glands) are inactive and mating can only occur when males are given juvenile hormone treatments[9]. Recently, parallel increase of in vitro JH biosynthesis and of pheromone responsiveness were found in newly emerged males of *Pseudaletia unipuncta*[10].

In the black cutworm, mating does not occur until a few days after eclosion[11], leaving open the possibility of an endocrine control of sexual events as suggested by Barth[12]. To explain this delay in reproductive processes and the ecological adult diapause of the black cutworm we proposed that JH may be involved in the maturation of pheromone communication and sexual behaviour in this noctuid. Using a strong JH mimic, fenoxycarb[13], we showed that in the female JH seems to be involved in the control of calling and mating[14]. We therefore used the same JH mimic and KK-42, a JH antagonist[15,16], together with surgical treatment, to test the ability of treated males to respond to the female sex pheromone in wind tunnel experiments.

In females of Lepidoptera, production and release of the pheromone biosynthesis activating neurohormone (PBAN) is necessary for pheromone production[17]. Allatectomy in female *Pseudaletia unipuncta* suppresses pheromone production. However, a partial recovery of sexual activity is observed in allatectomized females after injection of head extracts containing the PBAN[1]. The authors concluded that JH could modulate pheromone production and release either through the PBAN axis or directly via the pheromone gland. PBAN-like activity has also been detected in males of Lepidoptera but its role remains unknown[17]. Therefore we injected synthetic PBAN into allatectomized black cutworm males. Their responses to the pheromone blend were measured in a wind tunnel.

Materials and methods

Larvae of the black cutworm were reared on an artificial diet according to Poitout and Buès[18]. Boxes of pupae were observed each day for adult emergence which was then considered as day-0 of adult life. Males and females were held separately in plastic boxes in two different chambers and had access to 20% sucrose solution.

A flight tunnel derived from J. J. Turgeon et al.[3] was used according to R. Causse et al.[19]. Males were kept individually in plastic boxes under a 16L:8D inverted photoperiod and were transferred to the flight tunnel chamber a few minutes before being tested. Environmental conditions during the bioassay were held constant: 21 °C, wind speed of 1 m/s under red light not exceeding 3 lux. We used the synthetic pheromone blend which proved to be most efficient in the field (Z7-12Ac (20 μg), Z9-14Ac (5 μg)[20] and Z-11: 16Ac (10 μg)[19]. The septum was stored in a freezer between uses. It was placed upwind from the male on a plastic stand. After a few minutes during which males adjusted to the air flow, the pheromone was released and the behaviour of males was observed. Control males were always tested to assess the presence of the pheromone. Responsiveness of males to the pheromone blend was estimated using the following sequence: (0) no response, (CE) claspers extruded, (IF) interrupted flight, (CF) complete flight without landing, (L) landing on the attractive source.

Fenoxycarb treatments were performed ten days after pupation by a single topical application of a 10 μg/μl solution in acetone. Technical grade fenoxycarb was a gift from Dr. B. Mauchamp. Treatments with 1-benzyl-5-((E)-2, dimethyl-1, 7-heptadienyl) imidazole (KK-42) were given by injections (10 μg/μl in olive oil) ten days after pupation. KK-42 was a gift from Dr. Kuwano. Control treatments were performed by acetone topical applications and injections of olive oil respectively.

Allatectomies and sham-operations were performed according to Cusson and McNeil (see ref. 1). CA were implanted into allatectomized males on day-1 post-emergence. Donors were day-3 control males. A small opening was made in the abdomen of the operated male and one pair of CA gently pushed inside with a pair of fine forceps. Electroantennogram recordings were performed on living males according to Renou et al.[21]. Decreasing dilutions of the main active pheromone component (Z7-12Ac) were prepared in hexane. One microlitre of the appropriate dilution was deposited on a filter paper inserted in a glass cartridge. During experiments, the cartridge was introduced into a branch of a Y-shaped tube. A purge stream of humidified air (1.5 l/min) was continuously blown on the antenna through the other branch of the tube. Stimulations were achieved by blowing a puff of air (0.5 s; 0.5 l/min) through the cartridge. Increasing doses of Z7-12Ac were tested successively 3 times each.

Synthetic *Heliothis zea* PBAN (Hez-PBAN)[22] was purchased from Neosystem Laboratories (Strasbourg, France). Preliminary tests were performed on decapitated females of European corn borer moths to assess biological activity according to Sreng et al.[23]. Two microlitres of 10^{-6} M PBAN in distilled water were injected at day-3 into allatectomized males during the first half of the scotophase. The males were tested for pheromone response in the wind tunnel three hours later.

Results and discussion

Newly emerged males did not respond to a synthetic pheromone blend in a wind tunnel until day-3 (fig. 1). Using a potent JH agonist, fenoxycarb, we showed that day-0 sexually immature males, topically treated with fenoxycarb, responded positively to the synthetic pheromone (fig. 2). By contrast, day-3 males injected with a JH antagonist, KK-42, showed a significantly lower reaction to the pheromone (fig. 3). Control males and olive oil injected males showed good responses. It is thus possible to counteract the normal pheromonal response of black cutworm males by using an insect growth regulator agonist or antagonist.

To assess whether male sexual behaviour is controlled by JH, CA were removed from freshly emerged males.

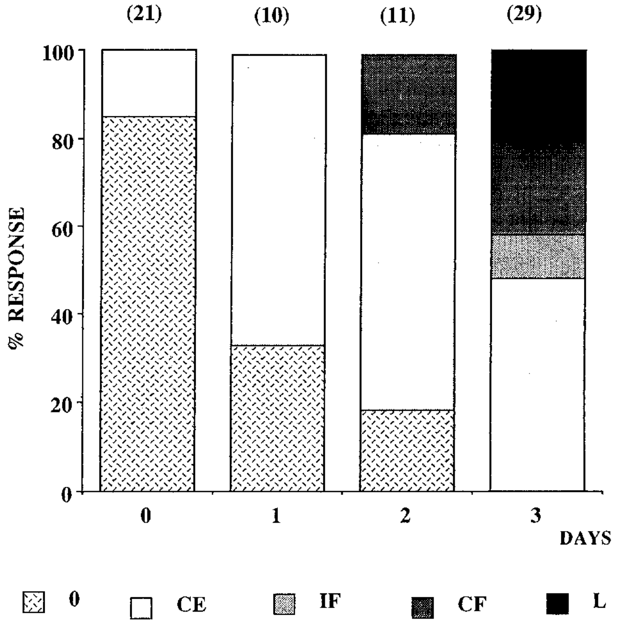

Figure 1. Effects of age in adult life on pheromone responsiveness of males of the black cutworm. 0, no response; CE, claspers extruded; IF, interrupted flight; CF, complete flight; L, landing on the source. ( ): number of insects.

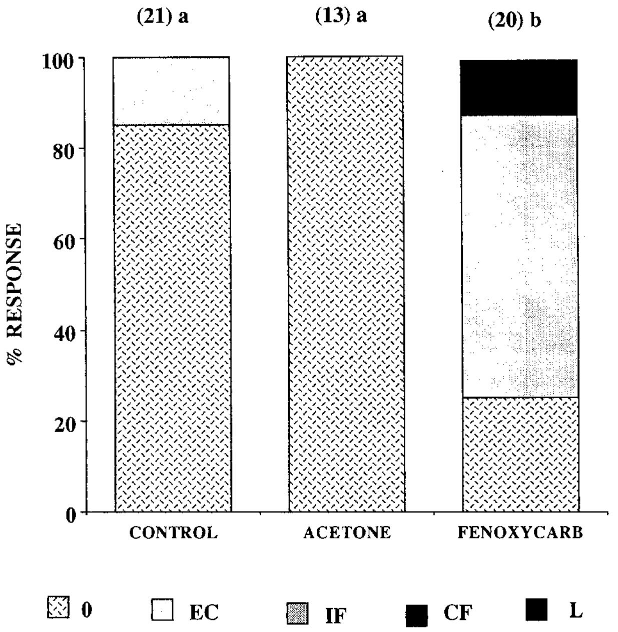

Figure 2. Effects of topical applications of a JH agonist, fenoxycarb (10 µg of fenoxycarb in 1 µl of acetone) on pheromone responsiveness of day-0 males of the black cutworm. 0, no response; CE, claspers extruded; IF, interrupted flight; CF, complete flight; L, landing on the source; ( ): number of insects. Bars followed by the same letter are not significantly different (X^2-test among proportions of non-responding and responding insects, $p = 0.05$).

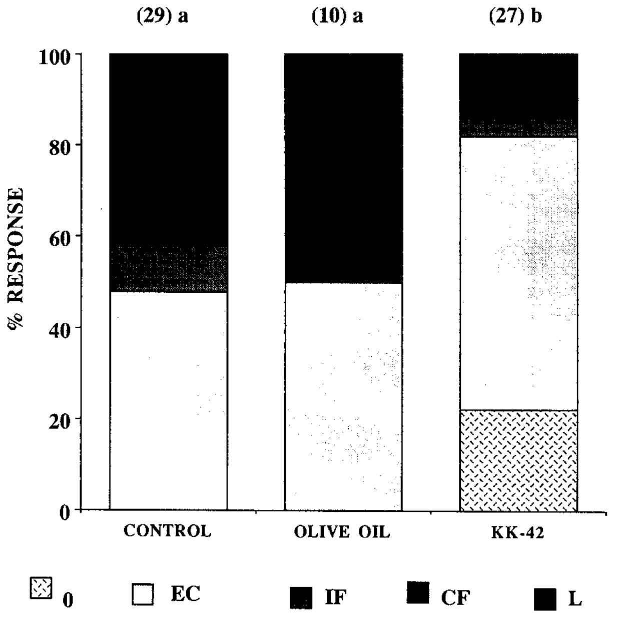

Figure 3. Effects of a JH antagonist, KK-42 (10 µg of KK-42 in 1 µl of olive oil injected half-way through the nymphal life) on pheromone responsiveness of day-3 males of the black cutworm. 0, no response: CE, claspers extruded; IF, interrupted flight; CF, complete flight; L, landing on the source; ( ): number of insects. Bars followed by the same letter are not significantly different (G-test, $p = 0.05$, CE and IF scores and CF and L scores were pooled).

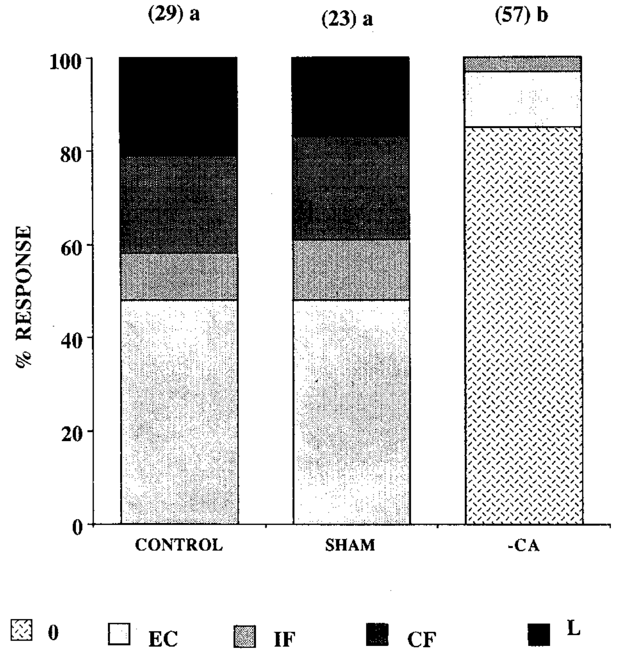

Figure 4. Effects of allatectomy (−CA) and sham operation (Sham) on pheromone responsiveness of black cutworm males. Wind tunnel experiments were performed at day-3 post-emergence. 0, no response, CE, claspers extruded; IF, interrupted flight; CF, complete flight; L, landing on the source; ( ): number of insects. Bars followed by the same letter are not significantly different (G-test, $p = 0.05$, CC and IF scores and CF and L scores were pooled).

We then tested the responsiveness of these males to pheromone blend in the flight tunnel. Allatectomy inhibited pheromone responsiveness of male black cutworm, whereas all control and sham-operated insects responded to the pheromonal stimulus (fig. 4). Pheromone responsiveness of allatectomized males was restored after implantation of active male CA (fig. 5).

Because allatectomy inhibited the responses to pheromone in the wind tunnel, we measured the antennal sensitivity to the main pheromone component (Z7-12:Ac) of allatectomized 3-day old males and compared it with that of 0-day old control males. No statistical difference in the amplitude of the EAG response was seen between operated and control insects, showing that allatectomy has no effect on the sensitivity of the pheromone receptors (table).

Allatectomized males of the black cutworm do not respond to a pheromone blend despite their ability to detect the female sex attractant through the antennae. Antennae from newly emerged males exhibited a normal sensitivity to Z7-12:Ac, but males of the same age do not respond to the pheromone blend in the flight tunnel (table). Thus, although JH did not affect the detection of the sex pheromone by the antennal receptors, it could modulate the processing of the sensory input and the elaboration of the motor response by the moth central nervous system.

Injections of Hez-PBAN into allatectomized males showed a partial recovery of pheromone responsiveness (fig. 5). Therefore we suggest that a male PBAN-like

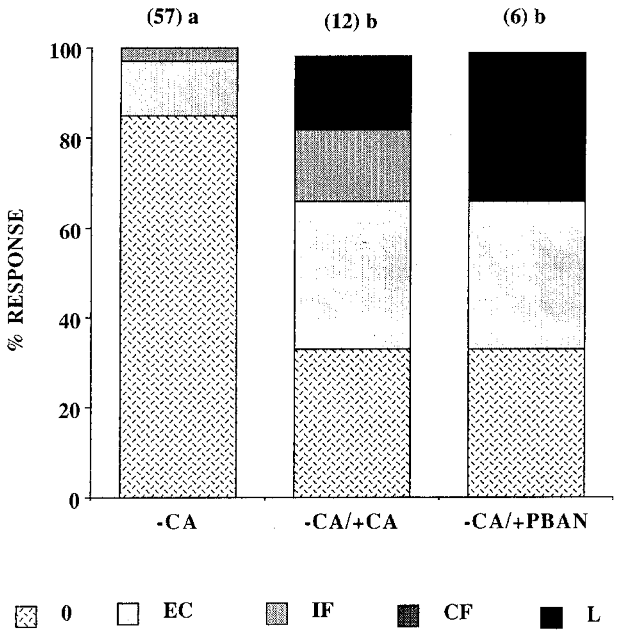

Figure 5. Effects of CA reimplantation and synthetic PBAN (10^{-6} M) on pheromone responsiveness of allatectomized males of black cutworm. Wind tunnel experiments were performed at day-3 post emergence. 0, no response; CE, claspers extruded; IF, interrupted flight; CF, completed flight; L, landing on the source; ( ): number of insects. Bars followed by the same letter are not significantly different (G-test, $p = 0.05$, CE and IF scores and CF and L scores were pooled).

Table. Effect of allatectomy (−CA) on the EAG responses to a low and a high dose of the main pheromone component of the black cutworm. There were no statistically significant differences among EAG amplitudes (Kruskal-Wallis test) for a given stimulus concentration

Treatment	n	EAG response (mV ± s.d.)	
		10 ng	500 ng
−CA	3	3.7 ± 0.7	5.3 ± 0.5
0-day	2	3.6 ± 1.1	5.7 ± 0.4
3-day	3	3.7 ± 0.1	5.8 ± 0.6

substance could modulate pheromone responsiveness and that its production or release could be controlled by the CA.

Females have been shown to synchronize their reproductive strategy to climatic conditions, so as to increase their changes of producing surviving progeny. Males also show an endogenous mechanism which enables them to adapt their sexual potential to female reproductive development and to abiotic factors. External factors such as photoperiod and temperature would act on the brain, triggering, through allatotropic factors, JH biosynthesis leading to the maturation of sexual behaviour. In autumnal conditions, males lacking the hormonal stimulus would fly southwards, unaffected by pheromone-baited traps. This is in keeping with the 'oogenesis-flight syndrome' proposed by Johnson[24] in which migrating females show an arrest of ovarian development during migration.

Maturation of sex pheromone responsiveness could therefore play an important role in adjusting male competence for mating to female sexual development.

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