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Brain-independent development in the moth *Sesamia nonagrioides*

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ABSTRACT

The caterpillars of *Sesamia nonagrioides* developing under long-day (LD) photoperiod pupate in the 5th or 6th instar whereas under short day (SD) conditions they enter diapause and undergo several extra larval molts. The diapause is terminated within 1–3 instars upon transfer of SD larvae to the LD conditions. Brain removal from the 6th instar larvae promotes pupation followed by imaginal development; however, one third of the SD larvae and 12% of the LD larvae debrained at the start of the instar first undergo 1–2 larval molts. The incidence of larval molts is enhanced by the brain implants. Exclusively pupal molts occur in the LD larvae debrained late in the 6th instar. Decapitation elicits pupation in both LD and SD larvae, except for some of the 4th and 5th and rarely 6th instar that are induced to a fast larval molt. The pupation of decapitated larvae is reverted to a larval molt by application of a juvenile hormone (JH) agonist. No molts occur in abdomens isolated from the head and thorax prior to the wandering stage. Abdomens isolated later undergo a larval (SD insects) or a pupal (LD insects) molt. Taken together the data reveal that in *S. nonagrioides* (1) several larval molts followed by a pupal and imaginal molt can occur without brain; (2) an unknown head factor outside the brain is needed for the pupal–adult molt; (3) brain exerts both stimulatory and inhibitory effect on the corpora allata (CA); (4) larval molts induced in CA absence suggest considerable JH persistence.

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1. Introduction

Since the time of Kopeć (1922) it has repeatedly been demonstrated that the development of insects is governed by the brain. Numerous studies performed in Lepidoptera showed that brain removal before a critical period prevented molting. Williams (1947) discovered in the silkmoth *Hyalophora cecropia* that brain translated environmental changes in photoperiod into a neurohormonal signal that stimulated molting hormone (= ecdysteroids) production from the prothoracic glands (PG). Brain neurosecretion was identified as prothoracicotropic hormone, PTTH (Kataoka et al., 1987). PG regulation proved more complex (Gilbert et al., 2002; Yamanaka et al., 2006; Watanabe et al., 2007) but pivotal role of brain is obvious from the cessation of development in the decapitated or debrained larvae.

The switch from larval development to metamorphosis is primarily controlled by juvenile hormone (JH) secretion from the corpora allata (Wigglesworth, 1934). Corpora allata (CA) of Lepidoptera were found to be hormonally regulated by the brain (Granger and Sehnal, 1974; Sehnal and Rembold, 1985). The role of brain in CA regulation was examined in a number of species and several allatotropic and allatostatic factors were isolated (Audsley et al., 2008). JH titer in many caterpillars is further regulated by a specific esterase that occurs in the last larval instar and renders JH inactive by cleaving off the methyl ester group (Hammock, 1985).

The Mediterranean corn borer, *Sesamia nonagrioides*, is a noctuid that produces 2–4 generations per season. Fully grown larvae of the late summer generation bore into the maize hypocotyl for overwintering and their pupation is postponed until next spring (Galichet, 1982). The hibernation was recognized as diapause induced by the short photoperiod (Eizaguirre and Albajes, 1992) and regulated by JH (Eizaguirre et al., 1998). In larvae that develop under long day conditions, JH titer in the hemolymph drops from about 20 nM in the 4th and 5th instar to undetectable level in the 6th instar when the insects pupate (Eizaguirre et al., 2005). Under the short day conditions, the JH titer is about 60 nM in the 4th and 5th instars and up to 20 nM in the 6th and subsequent extra larval instars that characterize the diapausing insects. The titer of total ecdysteroids in the 4th and 5th instars is similar in the diapause-destined and the non-diapausing larvae; in the 6th instar the titer raises to a small peak of 0.2 μg/ml hemolymph on day 5 and to a larger peak on days 7–9. This second peak reaches 0.6 μg/ml in the pupating larvae but less than 0.3 μg/ml in the diapausing ones (Eizaguirre et al., 2007).

Effects of hormone agonists administered to the larvae of *S. nonagrioides* suggested that JH and ecdysteroids affect each other's concentration in a way that gears subsequent development to

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perfect realization of one of the three programs: larval growth, diapause, and pupation, respectively. Mixed modes, indicating a failure of the regulatory mechanisms, are limited to the development of larval–pupal intermediates after application of JH agonist in the second third of the last larval instar (Eizaguirre et al., 2005). The stability of developmental programs indicated that a higher control center – presumably the brain – integrates environmental signals such as the photoperiod with those from the internal milieu (hormone titers, body size, etc.), "selects" the mode of development, and sends appropriate commands to the endocrine glands. This paper is the first attempt to elucidate regulatory mechanisms that ensure perfect realization of the chosen developmental program in this species. The tuning of regulatory centers to realization of a certain program (larval, diapause, or pupation) is called here "programming". It should be distinguished from the developmental programming of the target tissues that is referred to as "commitment" (Riddiford, 1976).

2. Materials and methods

2.1. Insects and their rearing

The culture of *S. nonagrioides* was established from insects collected in central Catalonia and reared on a semi-artificial diet at 25 °C as described by Eizaguirre et al. (1994). The age of larvae was specified in respect to the instar and the number of days after ecdysis; for example, L5d6 denotes larvae of the 5th instar, 6 days after the ecdysis. Larvae kept under the long day (LD) photoperiod of 16:8 h light:dark cycle pupated in the 5th (about 20%) or the 6th larval instar. Those going to pupate in the 5th instar were recognized and discarded on day 4 of this instar when the remaining larvae showed clear signs of larval apolysis. After ecdysis into the 6th instar the LD larvae fed for about 6 days (maximal weight was reached on day 5), wandered on day 7, underwent pupal apolysis on days 8–9, and ecdysed to pupae one day later. By contrast, larvae grown since hatching under the short day (SD) photoperiod of 12:12 h light:dark cycle never pupated in the 5th or 6th instar but entered diapause characterized by up to ten extra larval molts occurring in irregular intervals (Eizaguirre et al., 1994; Fantinou et al., 1996; Gadenne et al., 1997). Our experiments included alternation of the two photoperiods in L6d2. This age was chosen because treatments with JH agonist indicated that the tissues of LD larvae could still realize a perfect larval molt. The commitment for pupal molt was detected in some body regions in 40% of the L6d4 larvae (Eizaguirre et al., 2005).

2.2. Brain removal and implantation

The role of brain was examined by means of decerebration and brain re-implantation in the L6d1 and L6d5 larvae. We assumed, and the experiments confirmed, that the newly ecdysed L6d1 were developmentally flexible, while the L6d5 larvae, which experienced a small ecdysteroid peak (Eizaguirre et al., 2007), were partially committed to a certain type of molt. Larvae were anaesthetized by submerging in water for 2–3 h, blotted on absorbent paper, and fixed with dorsal side up in a paraffin-lined Petri dish by means of pins. Integument between the head capsule and the pronotum was stretched and cut with a scalpel. The head was bent forward and the partly exposed brain was removed with a pair of forceps's. Inspection of the dissected brain confirmed that the corpora cardiaca–corpora allata (CC–CA) complex remained in situ. Some of the debrained larvae received implants of brains taken from donors of the same age and either of the same or of the opposite photoperiodic experience than the recipients. Brains (without CC–CA) were dissected under insect saline from the cut-off heads and within 30 min implanted (two brains per specimen) into recipient larvae through a V-shaped slit in the integument on the dorso-lateral side of the abdomen. Following insertion of the implant, the V-flap of the integument was placed back, the wound was wiped, and the insects were kept individually and without food in a refrigerator for 2 h before return to appropriate rearing conditions. The debrained larvae without or with the implants did not feed but crawled when touched. They were kept in vials supplied with a small amount of diet to maintain humidity.

In the sham operated insects we verified that injuries mimicking the brain removal and brain implantations did not alter the type of development determined by the photoperiod. The implantation procedure (anesthesia in water, injury similar to the brain removal, insertion of an inert implant, and recovery in refrigerator) performed at L6d1 extended the length of the intermolt period by about one day. The extension was shorter in the L6d2 and L6d5 insects.

2.3. Decapitated larvae and isolated larval abdomens

Anaesthetized larvae were ligated with a thin cotton thread either behind the head or across the mesothorax. The body part anterior to the ligature was cut off. Body constriction behind the head yielded decapitated larvae that lacked the major neuroendocrine center brain–CC–CA but contained the prothoracic glands (PG). Neither of these organs was present in the isolated abdomens obtained by ligating the larvae across mesothorax. Both the decapitated larvae and the isolated abdomens were kept in vials supplied with a small amount of diet that provided air humidity. The insects were motionless but responded to tactile stimuli by body wriggling. The molts of decapitated larvae and isolated abdomens usually advanced until the stage of molting fluid resorption but the exuvia was fully shed off in a few insects only.

2.4. Application of juvenile hormone agonist

Juvenile hormone agonist methoprene (ethyl 3,7-dimethyl-11-methoxydodeca-2,4-dienoate) was obtained from the Zoecon Corporation (Palo Alto, CA) as >97% trans, trans, S isomer in 1994 and thereafter stored in a freezer. Tests with *Galleria mellonella* larvae showed that it had retained biological activity and was therefore used in the present study. L6d1 larvae of *S. nonagrioides* were decapitated and one day later treated topically with 2 μl acetone containing 1, 0.1, 0.01, 0.001, and 0 μg methoprene. One group of larvae treated with 0.01 μg received this dose again 3 and 6 days later (the last treatment was not applied to larvae that had already molted).

2.5. Statistical evaluation of the results

Experimental insects were checked daily. Mortality due to surgical manipulations occurred within 1–3 days and never exceeded 10%. Dead larvae were not considered in treatment evaluations and are not included in the "total number of insects" shown in the tables and figures. When the number of individuals permitted, the time to the first larval ecdysis or to the pupal apolyses was analyzed by one-, two- or three-way ANOVA, depending on the number of factors considered. LSD test was used to compare durations of the intermolt periods.

3. Results

3.1. Photoperiodic determination of metamorphosis versus diapause

About 20% of larvae grown since hatching under LD conditions pupated in the 5th instar and were discarded. All

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