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Role of the Corpus Allatum in the Control of Reproductive Diapause in the Male Blow Fly, *Protophormia terraenovae*

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ABSTRACT— Male *Protophormia terraenovae* showed a long-day photoperiodic response at 25°C with respect to the induction of mating behavior. Male adults transferred from long-day to short-day conditions at emergence showed no mating behavior. When the corpus allatum (CA) was removed 6–8 hr or 1 day after adult emergence under long-day conditions, about 60% of males showed mating behavior. When the CA was removed 2 days after adult emergence, however, most males showed mating behavior similarly to the intact controls. Application of a juvenile hormone analog increased the proportion of mating behavior from about 60 to 100% in males allatectomized 6–8 hr after adult emergence under long-day conditions, and from 0 to about 30% in intact males transferred to short-day conditions at adult emergence. Thus, the endocrine control of diapause in males is different from that in females in which the CA plays a predominant role. Male adults responded to photoperiod and showed mating behavior even after the CA was removed. The mating behavior is controlled also by a factor or factors other than the CA with respect to diapause.

INTRODUCTION

Inactivity of the corpus allatum (CA) in secreting juvenile hormone (JH) has been shown to be the key factor for reproductive (adult) diapause in many insect species (Denlinger, 1985). Although hormonal control of reproductive diapause has been less well studied in males than in females, the same endocrine mechanism for reproductive diapause has been proposed for both sexes in many species where the CA plays the key role (Pener, 1992). However, removal of the CA has no effect on the active sexual status in nondiapause male adults of the linden bug, *Pyrhocoris apterus* or the Colorado potato beetle, *Leptinotarsa decemlineata* (Žďárek, 1968; Thibout, 1982).

The leading role of the CA in the control of reproductive diapause in females has been demonstrated in several flies also (Kambysellis and Heed, 1974; Saunders *et al.*, 1990; Agui *et al.*, 1991; Burks *et al.*, 1992; Kim and Krafur, 1995; Matsuo *et al.*, 1997). Females of the blow fly, *Protophormia terraenovae*, enter reproductive diapause under short-day conditions (Numata and Shiga, 1995). In nondiapause adults raised under long-day conditions, removal of the CA suppresses ovarian development, and application of a juvenile hormone analog (JHA), methoprene, recovered ovarian development in both diapause and allatectomized nondiapause adults (Matsuo *et al.*, 1997). Male adults of *P.*

terraenovae also respond to photoperiod and do not show mating behavior under short-day conditions. In the present study, we examined the role of the CA in the development of mating behavior in male *P. terraenovae* following application of JHA and various surgical procedures.

MATERIALS AND METHODS

Insects

A laboratory culture of *P. terraenovae* that originated from adults collected in Obihiro City, Japan was used. This culture was maintained under a long-day photoperiod of L:D 18:6 hr (18 hr light and 6 hr darkness) at 25±1°C. Insects for the experiments were reared under a long-day or a short-day photoperiod (L:D 12:12 hr) at 25±1°C, as previously reported (Numata and Shiga, 1995). Adults were collected within 2 hr after emergence (day 0), and 7–13 individuals of either sex were reared in plastic pots (15 cm in diameter, 9 cm in depth) covered with nylon net. They were fed on sucrose and water, and beef liver was supplied on day 3.

Observation of male mating behavior

On day 5, one virgin male and one virgin female were introduced into a plastic pot (15 cm in diameter, 9 cm in depth) and their behavior was observed for 30 min. Only females reared under L:D 18:6 hr were used, because most females develop their ovaries in 5 days under these conditions (Matsuo *et al.*, 1997). The mating behavior of *P. terraenovae* was similar to that reported in the house fly, *Musca domestica* (Tobin and Stoffolano, 1973). A male landing on the dorsum of a female was regarded as attempting copulation. A male completing genital connection with a female was regarded as succeeding in copulation. Copulation continued for more than 10 min in all cases tested. Statistical analysis was performed by Tukey type multiple comparison test for proportions (Zar, 1996, p. 560–562).

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JHA application

A JHA, methoprene, was obtained commercially as Manta® (Otsuka Chemical Co.). The solvent was evaporated under a flow of N₂. Then, methoprene was diluted to 0.63 mg/ml in acetone/peanut oil (9:1) solution, and 1 µl of diluted solution was applied topically onto the ventral thorax with a glass capillary (Ohtaki *et al.*, 1972). In controls, only the acetone/peanut oil solution was applied.

Surgical procedures

The following surgical procedures were performed in males. The neck membrane was exposed without anesthesia and was cut with fine forceps. (1) The CA was separated from the corpus cardiacum and hypocerebral ganglion complex (CCHG) with small scissors, and taken out with fine forceps. (2) The nervi corporis allati (NCA) was transected between the CCHG and the CA with small scissors. (3) The cardiac recurrent nerve (CRN) was severed at a posterior part close to the CCHG with small scissors. (4) After cutting the NCA and CRN, the CCHG was separated from the esophagus with small scissors, and taken out with fine forceps. In sham operation, only the neck membrane was incised.

RESULTS

Response to photoperiod in intact insects

First, we examined the effects of photoperiod on male mating behavior. When insects were continuously reared under long-day conditions, most males succeeded in copulation 5 days after adult emergence. Males transferred from long-day to short-day conditions at adult emergence showed no mating behavior similarly to those reared continuously under

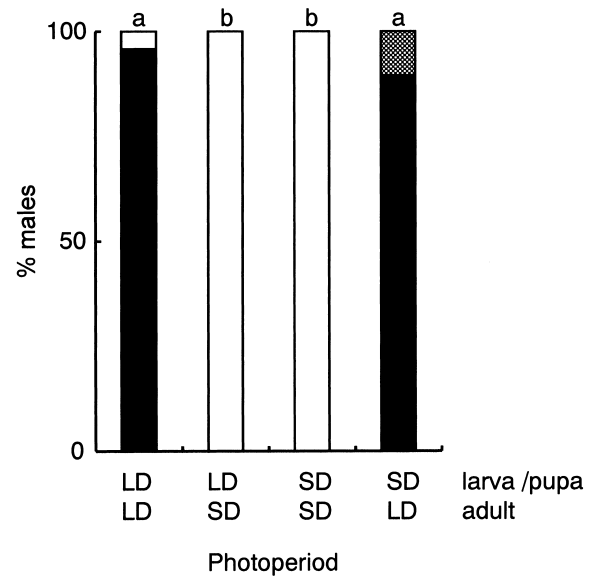


Fig. 1. Effects of photoperiod on mating behavior of male *Protophormia terraenovae*. Mating behavior of a male was observed for 30 min, 5 days after adult emergence with a reproductive female. LD, a long-day photoperiod of L:D 18:6 hr at 25°C; SD, a short-day photoperiod of L:D 12:12 hr at 25°C. Solid columns, successful copulation; shaded columns, copulation attempted but unsuccessful; open columns, no mating behavior. The values with the same letter above columns are not significantly different ($P > 0.05$, Tukey type multiple comparison for proportions of males showing mating behavior). $n = 19-25$.

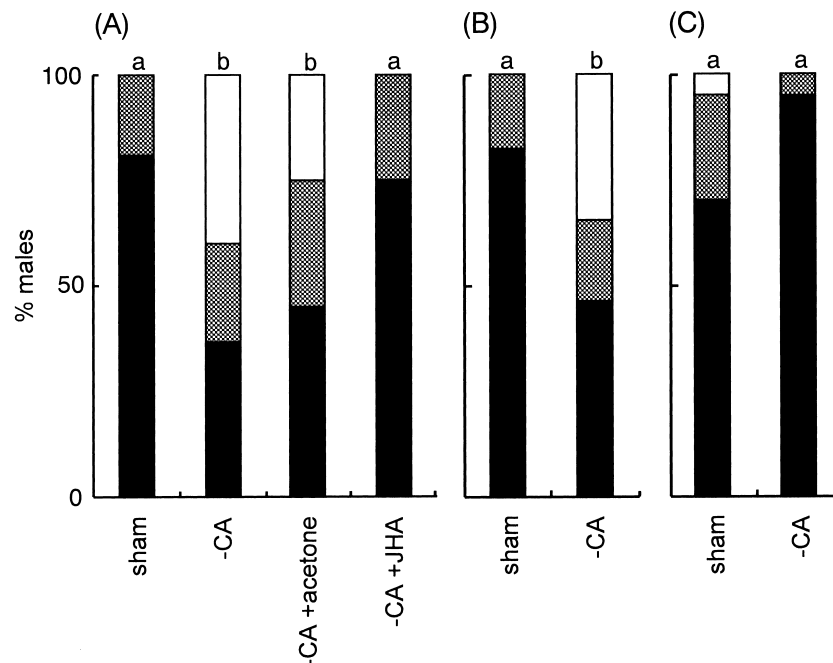


Fig. 2. Effects of allatectomy (-CA) on mating behavior and its recovery by a juvenile hormone analog in male *Protophormia terraenovae* reared under a long-day photoperiod (L:D 18:6 hr) at 25 °C. The corpus allatum was removed 6–8 (A), 22–24 (B) or 46–48 hr (C) after adult emergence. sham, sham operation; -CA +acetone, allatectomy followed by application of acetone/peanut oil solution; -CA +JHA, allatectomy followed by application of 0.63 µg of methoprene on days 1 and 3. Mating behavior of a male was observed for 30 min 5 days after adult emergence with a reproductive female. Solid columns, successful copulation; shaded columns, copulation attempted but unsuccessful; open columns, no mating behavior. The values with the same letter above columns are not significantly different ($P > 0.05$, Tukey type multiple comparison for proportions of males showing mating behavior). $n = 17-30$.

short-day conditions. However, all males attempted copulation and most of them succeeded it when they were transferred from short-day to long-day conditions at adult emergence (Fig. 1). Thus, photoperiodic conditions imposed during the first 5 days in the adult stage suffice to control mating behavior in males.

Effect of allatectomy and its recovery by JHA under long-day conditions

To examine the role of the CA in the induction of mating behavior in males, we removed the CA from males reared under long-day conditions, 6–8 hr, 1 day (22–24 hr) or 2 days (46–48 hr) after adult emergence. More than 95% of sham-operated males showed mating behavior similar to that in intact males. When the CA was removed 6–8 hr or 1 day after adult emergence, the proportion of males which attempted copulation was significantly lower than that in sham-operated controls, although about 60% of allatectomized males showed mating behavior (Fig. 2A, B). In contrast, allatectomy had no effect on mating behavior when the operation was performed 2 days after adult emergence (Fig. 2C). Thus, the CA activity within 48 hr after adult emergence was necessary to induce mating behavior in all males.

To verify that the decrement of mating activity by allatectomy was due to the deficiency of JH, we applied a

JHA to allatectomized male adults. The CA was removed 6–8 hr after adult emergence from males reared under long-day conditions, and 0.63 μg JHA was applied on days 1 and 3. Application of acetone/peanut oil solution had no effect, although JHA application induced mating behavior in all allatectomized males (Fig. 2A).

JHA application, allatectomy and transection of the NCA under short-day conditions

As JHA application and transection of the NCA induce complete ovarian development in diapause female adults of *P. terraenovae* (Matsuo *et al.*, 1997), we examined the effects of these treatments in males. First, we applied 0.63 μg JHA on day 1 and 3 to males transferred from long-day to short-day conditions at adult emergence. Males treated with acetone/peanut oil solution showed no mating behavior similarly to untreated controls. After JHA application, however, about 30% of males showed mating behavior (Fig. 3).

Then, we removed the CA or transected the NCA 6–8 hr after adult emergence in males reared under long-day conditions and transferred them to short-day conditions. Sham-operated and allatectomized males showed no mating behavior similarly to intact controls. After transection of the NCA, about 30% of males showed mating behavior. However, none of the males subjected to these treatments succeeded in copu-

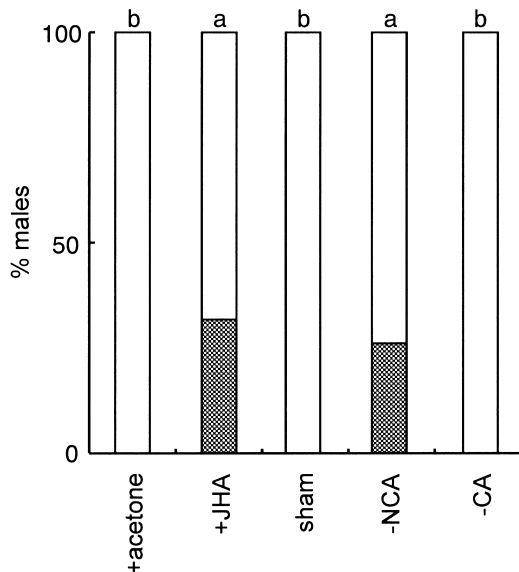


Fig. 3. Effects of a juvenile hormone analog (JHA), transection of the nervi corporis allati (–NCA), and allatectomy (–CA) on mating behavior of male *Protophormia terraenovae* reared under a long-day photoperiod (L:D 18:6 hr) and transferred to a short-day photoperiod (L:D 12:12 hr) at adult emergence (25°C). +acetone, application of acetone/peanut oil solution; +JHA, application of 0.63 μg methoprene on days 1 and 3; sham, sham operation for –NCA. Surgical procedures were performed 6–8 hr after adult emergence. Mating behavior of a male was observed for 30 min 5 days after adult emergence with a reproductive female. Shaded columns, copulation attempted but unsuccessful; open columns, no mating behavior. The values with the same letter above columns are not significantly different ($P > 0.05$, Tukey type multiple comparison for proportions). $n = 20–23$.

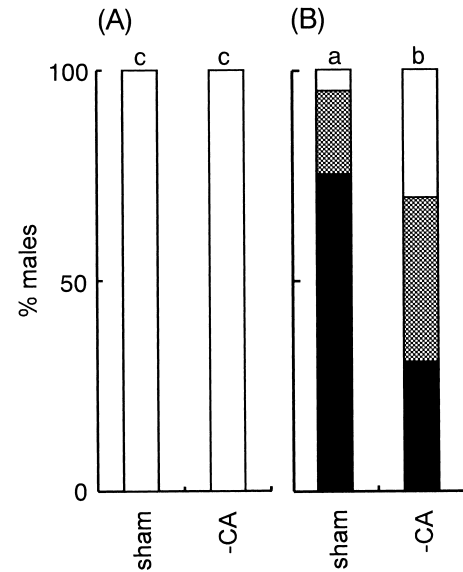


Fig. 4. Effects of allatectomy (–CA) on mating behavior of male *Protophormia terraenovae* reared continuously under a short-day photoperiod (A), or transferred from a short-day (L:D 12:12 hr) to a long-day photoperiod (L:D 18:6 hr) at adult emergence (B) at 25°C. The corpus allatum was removed 6–8 hr after adult emergence. sham, sham operation. Mating behavior of a male was observed for 30 min 5 days after adult emergence with a reproductive female. Solid columns, successful copulation; shaded columns, copulation attempted but unsuccessful; open columns, no mating behavior. The values with the same letter above columns are not significantly different ($P > 0.05$, Tukey type multiple comparison for proportions of males showing mating behavior). $n = 20–23$.

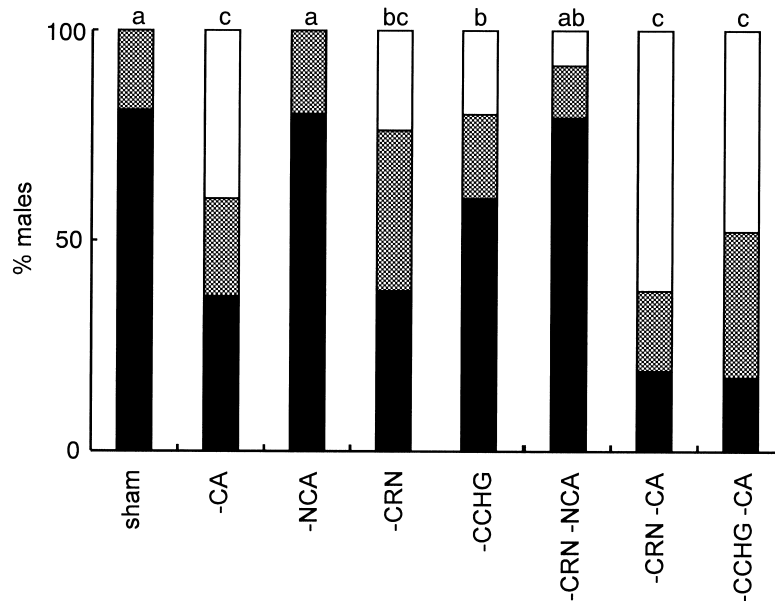


Fig. 5. Effects of various surgical operations on mating behavior of male *Protophormia terraenovae* reared under a long-day photoperiod (L:D 18:6 hr) at 25°C. Surgical procedures were performed 6–8 hr after adult emergence. sham, sham operation; –CA, allatectomy; –NCA, transection of the nervi corporis allati; –CRN, transection of the cardiac recurrent nerve; –CCHG, removal of the corpus cardiacum and hypocerebral ganglion complex. Mating behavior of a male was observed for 30 min 5 days after adult emergence with a reproductive female. Solid columns, successful copulation; shaded columns, copulation attempted but unsuccessful; open columns, no mating behavior. The values with the same letter above columns are not significantly different ($P > 0.05$, Tukey type multiple comparison for proportions of males showing mating behavior). $n = 20–30$.

lation (Fig. 3). Thus, neither JHA application nor transection of the NCA induced complete mating activity in males.

Allatectomy of insects reared under short-day conditions in the larval and pupal stages

Even when the CA was removed 6–8 hr after adult emergence under long-day conditions, about 60% of males showed mating behavior (Fig. 2A). We suspected that some adults subjected to long-day conditions in the larval and pupal stages might secrete sufficient amounts of JH for induction of mating behavior within 6–8 hr after emergence. Assuming that such early secretion of JH does not occur under short-day conditions, we removed the CA 6–8 hr after emergence from males reared under short-day conditions in the larval and pupal stages. Then, these males were kept under the same photoperiodic conditions or transferred to long-day conditions. Under short-day conditions, neither sham-operated nor allatectomized males showed mating behavior (Fig. 4A). After transferred to long-day conditions, about 70% of allatectomized males showed mating behavior similarly to those reared continuously under long-day conditions (Fig. 4B).

Various surgical operations under long-day conditions

We examined the effects of surgeries to the brain-retrocerebral complex 6–8 hr after adult emergence on the mating behavior of males reared continuously under long-day conditions. Transection of the NCA had no effect. Transection of the CRN and removal of the CCHG significantly reduced the proportion of males showing mating behavior.

Transection of both the NCA and the CRN resulted in a proportion of males showing mating behavior intermediate between those of sham-operated and CRN-transected insects, and the proportion of males completing copulation was significantly higher in males with the NCA and the CRN transected than in CRN-transected males ($P < 0.001$). The CCHG may reduce the CA activity by way of the NCA. Transection of the CRN or removal of the CCHG combined with allatectomy slightly reduced the proportion of males showing mating behavior as compared with allatectomy alone, although the decrease was not statistically significant (Fig. 5).

DISCUSSION

Pener (1974, 1986, 1997) listed the effects of the CA on male mating behavior in acridids, and concluded that male mating behavior is controlled by the CA in species with a reproductive diapause. In the bean bug, *Riptortus clavatus*, application of a JHA induced mating behavior in most diapause male adults (Numata and Hidaka, 1984). Among flies, the role of the CA in male mating behavior has been examined only in the dung fly, *Scatophaga stercoraria*. Allatectomized males of this species show no mating behavior although the control by the CA is not related to reproductive diapause but to nutritional conditions (Foster, 1967). Male adults of *P. apterus* showed high mating activity even after removal of the CA under diapause-averting conditions, although implantation of an active CA induced mating behavior under diapause-inducing conditions (Žďárek, 1968). In *L.*

decemlineata neither removal of the CA under diapause-averting conditions nor application of JH or JHA under diapause-inducing conditions had any effect on mating activity in males (Thibout, 1982). The degree of control by the CA in male mating behavior varies with species.

About 60% of *P. terraenovae* males showed mating behavior after CA removal within 1 day after adult emergence under diapause-averting conditions (Fig. 2A, B). Furthermore, application of a JHA induced mating behavior in about 30% of males with no successful copulation under diapause-inducing conditions (Fig. 3). Thus, inactivity of the CA can partially explain the suppression of male mating behavior in diapause, in contrast to the results in females showing that the CA plays a predominant role in the suppression of vitellogenesis during diapause (Matsuo *et al.*, 1997). In a strain of the migratory locust, *Locusta migratoria migratorioides* without reproductive diapause, allatectomy reduces but does not totally inhibit mating activity of males under gregarious conditions (Wajc and Pener, 1969; Pener *et al.*, 1972; Pener, 1976). They proposed the partial control of male mating behavior by the CA, but Barth and Lester (1973) regarded this depression of mating behavior to occur on a consequence of the general metabolic effects of reduced JH titer. We cannot exclude the possibility that the reduction of male mating behavior induced by allatectomy and recovered by JHA resulted from a general metabolic effect in *P. terraenovae*.

Male adults of *P. terraenovae* responded to photoperiod by inducing or suppressing mating behavior even after the CA was removed, whether they had been subjected to diapause-averting or diapause-inducing conditions in the larval and pupal stages (Figs. 2A, 3, 4). Therefore, the mating behavior is also controlled by a factor other than the CA with respect to diapause.

In *L. m. migratorioides*, electrocoagulation of the pars intercerebralis (PI) completely inhibits male mating behavior and implantation of active CA does not ameliorate this effect (Pener *et al.*, 1972). Therefore, the PI is necessary for induction of male mating behavior without intervention of the CA in this species. In female adults of *P. terraenovae*, removal of the PI, transection of the nervi corporis cardiaci and removal of the HGCC result in complete suppression of vitellogenesis (Toyoda *et al.*, 1999). In males also, transection of the CRN and removal of the CCHG decreased the mating activity (Fig. 5). Based on these results, we assumed that the deficiency of a factor from the brain or HGCC is responsible for the suppression of mating behavior in diapause without intervention of the CA. However, the strain of *L. m. migratorioides* used by Pener *et al.* (1972) has no reproductive diapause and the experiments in *P. terraenovae* were performed under diapause-averting conditions. Therefore, it is not clear whether these candidate factors are in fact suppressed in diapause males.

Furthermore, 40–50% of males showed mating behavior even after transection of the CRN with allatectomy or removal of both the CA and the CCHG under diapause-averting conditions (Fig. 5), whereas no mating behavior was observed

under diapause-inducing conditions (Fig. 1). Other factors are involved in the suppression of mating behavior in diapause male adults of *P. terraenovae*. Hodkova (1994) showed that in *P. apterus* removal of the PI induces mating behavior in diapause male adults, and concluded that a factor from the PI suppresses the behavior in diapause. Further studies should be performed to determine whether such a factor suppressing the mating behavior exists in *P. terraenovae*.

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