

# Inhibition of Juvenile Hormone During the Formation of the Spermatophore in *Blattella germanica* (L.) (Dictyoptera, Blattellidae)

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Allatectomy showed that Juvenile Hormone (JH) induced the accumulation of proteins in the accessory reproductive glands (ARG) of male *Blattella germanica*. During mating, the formation of the spermatophore and its transfer to the female was observed, and a selective depletion of most ARG proteins occurred in parallel, as shown by electrophoretic studies carried out at selected moments of the process. The synthetic activity of corpora allata incubated in vitro, measured at the same times, indicated that JH production is inhibited during the formation and transfer of the spermatophore. It is suggested that this inhibition may be necessary to start a new cycle of ARG maturation. © 1996 Wiley-Liss, Inc.

**Key words:** accessory reproductive glands, protein pattern, corpora allata, allatectomy, cockroaches

## INTRODUCTION

The ARG\* of male insects are mainly involved in the formation of the spermatophore (Kaulenas, 1992). In the cockroach *Blattella germanica*, ARG are extremely complex, and consist of a mass of tubules arranged in two principal groups (Khalifa, 1950; Ballan-Dufrançaïs, 1968; Piulachs et al., 1992). The first group comprises 4–6 long tubules (uricose glands) located in the apical part of the ARG. They serve as an organ of storage-excretion of uric acid (Roth, 1967; Cochran, 1985). The other group shows a mushroom-shaped body formed by up to 12 different categories of tubules or utricles (Feliubadaló et al., 1996), surrounding the seminal vesicles.

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\*Abbreviations used: ARG = accessory reproductive glands; CA = corpora allata; JH = juvenile hormone; kDa = kilodalton.

In previous papers (Piulachs et al., 1992; Bellés and Piulachs, 1992) we report that ARG of *B. germanica* undergo a steady increase in protein content during the first 10 days of adult life, which is paralleled by increasing rates of JH synthesis, as measured on CA incubated in vitro. In addition, we have shown that allatectomy reduces protein accumulation in the ARG, whereas the administration of exogenous JH restores normal development. These data suggest that JH induces the accumulation of proteins in the ARG.

In the present paper we describe (1) the effects of allatectomy on selected protein bands of the ARG pattern, (2) the dynamics of depletion of proteins corresponding to these bands during the formation of the spermatophore and that of refilling after its transfer to the female, and (3) the rates of JH synthesis by CA incubated in vitro which occurs in parallel. The working hypothesis was that increasing rates of JH synthesis would be necessary to refill the ARG tubules with proteins after the formation of the spermatophore. This was indeed the case, but an unexpected inhibition of JH synthesis was also observed during the spermatophore formation. The possible physiological meaning of this inhibition is discussed under the hypothesis that it may be necessary to start a new cycle of ARG maturation.

## MATERIALS AND METHODS

### Insects

Virgin males of *B. germanica* were from a colony fed on Panlab dog chow and water, and reared in the dark at  $30 \pm 1^\circ\text{C}$  and 60–70% r.h. For mating experiments, 12-day-old virgin males and 5-day-old virgin females were used.

### Protein Studies

ARG were dissected out as described elsewhere (Piulachs et al., 1992). Total soluble proteins were quantified according to Bradford (1976), using bovine serum albumin as standard. SDS-PAGE electrophoresis of ARG proteins was carried out using the method of Laemmli (1970) with 15% polyacrylamide slab gels. Electrophoresis was run at constant voltage (150 V) for 45 min, and gels were stained with Coomassie blue. Molecular markers were from Sigma, Madrid, Spain (SigmaMarker, Low Range: MW 6.5–66 kDa, and phosphorylase b from rabbit muscle: MW 97 kDa). At least 4 replicates of each electrophoretic study were carried out. Protein levels were estimated by densitometry of the gels with a Molecular Dynamics (Sunnyvale, CA) computing densitometer. Densitometry values were expressed as arbitrary absorbance units.

### Allatectomy and Juvenile Hormone Treatments

The corpora allata–corpora cardiaca complex was dissected out from freshly ecdysed adults (less than 5 h of imaginal life), and sham operations were carried out by removing the cervical sclerite and adjacent main tracheae. All operations were conducted on CO<sub>2</sub> anaesthetized specimens. JH III (Sigma), the native JH in *B. germanica* (Camps et al., 1987), was administered to allatectomized specimens in two doses (10 µg each), one after allatectomy

and the other 48 h later. The hormone was topically applied in acetone solution (2  $\mu$ l) on the ventral part of the abdomen. Controls received 2  $\mu$ l of acetone. Effects induced by allatectomy were checked on day 5 and 10 of adult life. Those induced by allatectomy plus JH treatment were studied on day 5.

### Measurement of Juvenile Hormone Synthesized In Vitro

Individual pairs of CA were incubated for 2 h in 100  $\mu$ l of TC 199 medium (Flow, Ayrshire, Scotland, UK), containing L-methionine (0.1 mM), Hank's salts, Hepes buffer (20 mM) plus Ficoll (20 mg/ml), to which L-[<sup>3</sup>H-methyl] methionine (Amersham, Buckinghamshire, UK) had been added to achieve a final specific activity of 7.4 GBq/mmol. Details of the method for JH III determination in vitro in *B. germanica* are given in Piulachs and Couillaud (1992).

## RESULTS

### Allatectomy and Proteins

The electrophoretic pattern of the ARG was first studied on day 5 of adult life in allatectomized specimens (Fig. 1a). The pattern showed four main bands: one corresponding to a molecular weight of about 65 kDa, another close to 45 kDa, and two more around 15 kDa (numbered 1 to 4, respectively). An additional, more variable band (not numbered) appeared around 24 kDa. These bands were markedly less intense in allatectomized than in intact males, especially bands 3 and 4. The patterns corresponding to sham-operated specimens and to allatectomized specimens treated with JH were, in general, comparable to controls. Those of allatectomized specimens treated with acetone were almost identical to those subjected to allatectomy alone.

When allatectomized specimens were studied on day 10 of adult life, the effects were much less apparent (Fig. 1c). Bands 1 to 4 were less intense than those observed in 5-day-old control males, but clearly more intense than those resulting from 5-day-old allatectomized specimens.

The densitometry of these gels allowed us to analyze the differences more precisely. In 5-day-old males (Fig. 1b), bands 3 and 4 were more affected by allatectomy (they were 80% less intense than in intact specimens) than bands 1 and 2 (70 and 60% less intense, respectively). In allatectomized males treated with JH, it was also apparent that bands 1, 2, and 3 gave similar values with respect to controls, whereas band 4 only showed about half the expected intensity. Data from the comparison of 5- and 10-day-old allatectomized specimens (Fig. 1d) agree with the qualitative observations made on the gel itself, and also indicate that the differences in the relative intensity of the four bands observed in allatectomized specimens on day 5 become almost negligible on day 10.

### Mating and Proteins

For mating studies, 12-day-old males were used. In order to study the state of the ARG during and after mating, the following physiological stages were established on males of the above age. A: virgin (control); B: some 50 min after the beginning of mating, spermatophore in process of formation, ARG

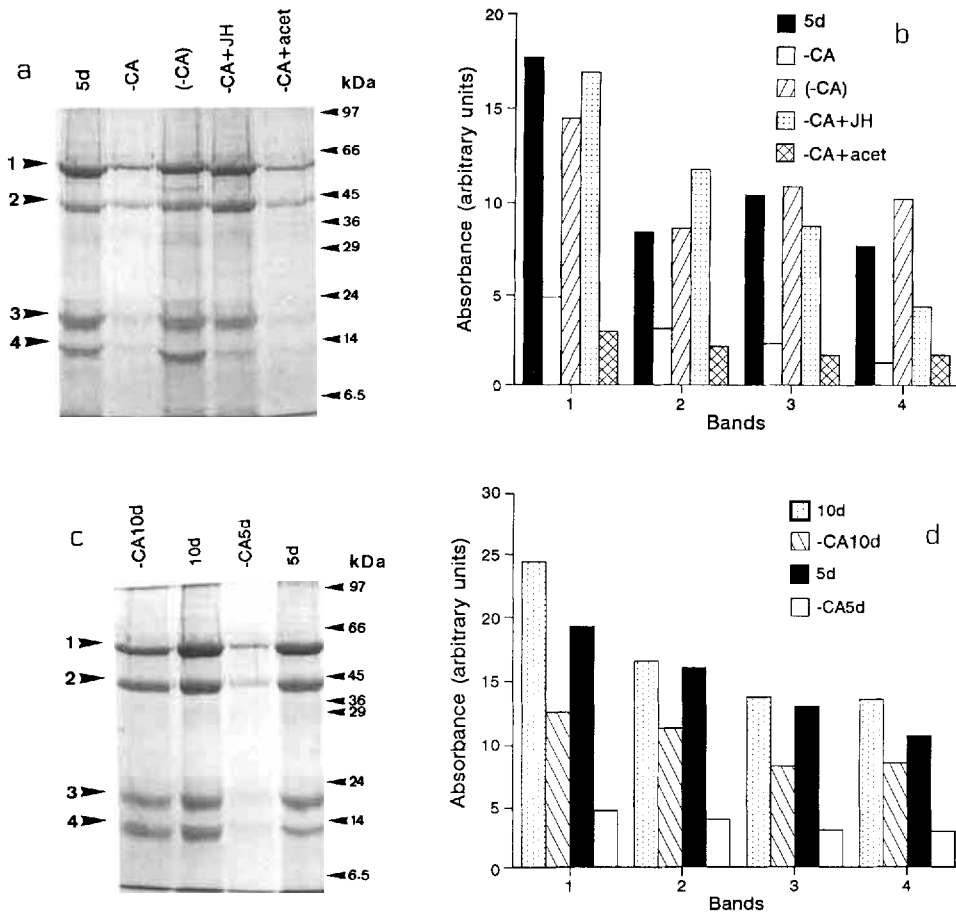


Fig. 1. Protein pattern (15% SDS-PAGE electrophoresis) of accessory reproductive glands of male *B. germanica*. **a**: Effects of allatectomy on day 0, measured on day 5 of adult life. **Lanes**: 5d, control; -CA, allatectomized; (-CA), sham-operated; -CA+JH, allatectomized and treated with juvenile hormone; -CA+acet, allatectomized and treated with acetone. **b**: Densitometry of the four main bands appearing in gel a. **c**: Effects of allatectomy on day 0, measured on days 5 and 10 of adult life. **Lanes**: -CA10d, 10-day-old allatectomized; 10d, 10-day-old control; -CA5d, 5-day-old allatectomized; 5d, 5-day-old control. **d**: Densitometry of the four main bands appearing in gel c. The arrowheads to the right indicate the molecular weight markers, and the four main bands studied, numbered 1 to 4, are indicated to the left. The equivalent of 15% of a gland was loaded in each lane.

with the utriculi slightly depleted and uricose glands full of secretion; C: some 10 min later, spermatophore formed but not transferred, utriculi empty and uricose glands slightly depleted; D: some 10 min later, spermatophore freshly transferred, utriculi and uricose glands empty; E: some 10 min later, male just after termination of mating; F: 6 h after termination of mating; G: 24 h later; H: 48 h later.

Total protein contents of the ARG in these stages (Fig. 2) showed a progressive decline during mating, and a steady recovery during the 2 days fol-

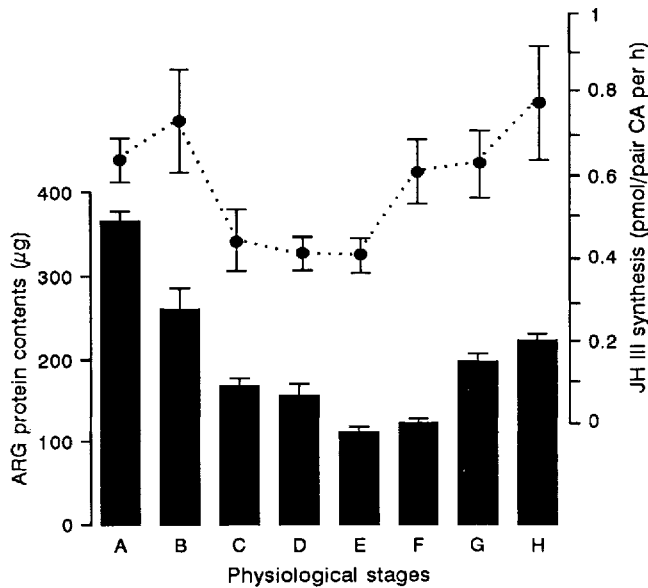


Fig. 2. Juvenile hormone synthesis by corpora allata incubated in vitro, and total protein contents of the accessory reproductive glands in male *B. germanica* during and after mating. Physiological stages: A, virgin (control); B, spermatophore in process of formation (50 min after the beginning of mating); C, spermatophore formed but not transferred (10 min later); D, spermatophore freshly transferred (10 min later); E, male just after termination of mating (10 min later); F, 6 h after termination of mating; G, 24 h later; H, 48 h later. Values are expressed as the mean  $\pm$  SEM (n = 6–38). In the measurements of juvenile hormone, values from stages D and E are significantly different from B, and stages C, D, and E are significantly different from H (Duncan's test,  $P < 0.05$ ).

lowing mating. The electrophoretic pattern of ARG in stages A, C, D, and E was then studied (Fig. 3a), and indicated that, whereas proteins corresponding to bands 1, 3, and 4 were suddenly depleted (they lost between 70 and 80% of intensity during spermatophore formation, Fig. 3b), those corresponding to band 2 decreased progressively, and only lost about 30% of intensity in the same period (Fig. 3b).

### Mating and Juvenile Hormone

Synthesis of JH by CA incubated in vitro was measured during and after mating at the same stages defined above. The results (Fig. 2) showed that the highest rates were found at the beginning of mating (about 0.7 pmol/pair CA per h), but they suddenly decreased thereafter, to maintain low values (about 0.4 pmol/pair CA per h) during the process of spermatophore transfer (stages C to E). Then, JH biosynthetic activity increased, and 48 h after mating the rates (about 0.7 pmol/pair CA per h) became similar to those found at the beginning of spermatophore formation.

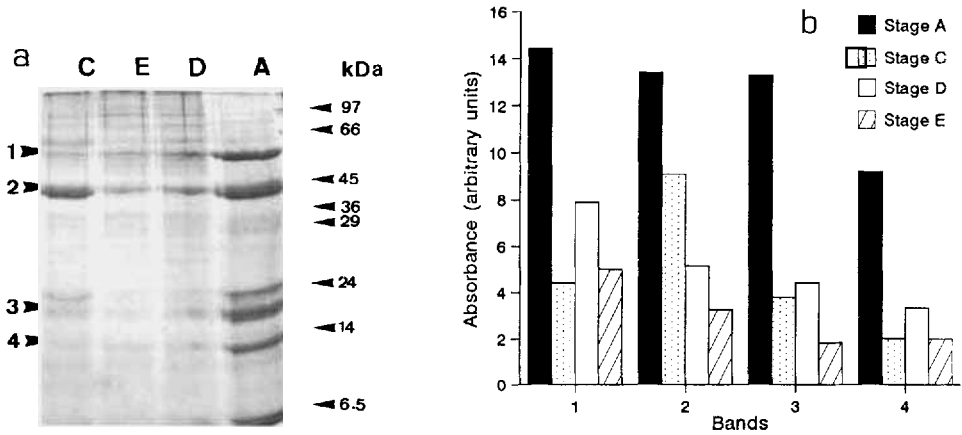


Fig. 3. **a:** Protein pattern (15% SDS-PAGE electrophoresis) of accessory reproductive glands (ARG) of male *B. germanica* in different physiological stages related with mating. **Lanes:** A, virgin (control); C, spermatophore formed but not transferred; D, spermatophore freshly transferred; E, just after termination of mating. The four main bands, numbered 1 to 4, are indicated to the left, and molecular weight markers are indicated to the right. The equivalent of 15% of a gland, or selected gland tubules, was loaded in each lane. **b:** Densitometric values corresponding to the four main bands of gel a.

## DISCUSSION

In previous contributions (Piulachs et al., 1992; Bellés and Piulachs, 1992) we reported that allatectomy in freshly ecdysed males of *B. germanica* slows the accumulation of proteins in the ARG. The electrophoretic studies reported here agree, in general, with our previous observations. Using 15% polyacrylamide gels (instead of 12%: Bellés and Piulachs, 1992), four main bands (numbered 1 to 4) were recognized in the electrophoretic pattern of ARG. When the effects of allatectomy were checked on day 5 of adult life, the intensity of these bands became reduced, and densitometric measurements indicated that the effect was slightly more apparent on bands 3 and 4. The protein pattern in allatectomized males that were treated with JH was similar to that of controls, except for band 4, which recovered only half the intensity of the respective controls. This suggests that other factors could be involved in inducing the synthesis of protein(s) corresponding to this band. These factors might be related with the corpora cardiaca, which was also dissected out during allatectomy, but not replaced with JH therapy. This hypothesis recalls the case of *Rhodnius prolixus*, where Davey and associates described that both JH and a neuropeptide affect protein synthesis in the ARG (Barker and Davey, 1983; Gold and Davey, 1989).

When the effects of allatectomy were checked on day 10 of adult life, they were less clear-cut than on day 5, which also agrees with our previous results based on total protein measurements of the ARG (Bellés and Piulachs, 1992). In 10-day-old males, the 4 main bands were less intense in allatectomized than in intact specimens, which indicates that the accumulation of the corresponding proteins was less efficient. However, considering allatec-

tomized specimens, it was apparent that these bands were more intense on day 10 than on day 5, which indicates that protein accumulation continues, albeit slowly, in the absence of CA.

The results agree with our previous observations (Piulachs et al., 1992) that allatectomy reduces protein accumulation rates in the ARG, rather than impairing that process irreversibly. In addition, long-term experiments (allatectomized males checked on day 10) suggest that no clear-cut differential effects of allatectomy occur at the level of selected proteins, although observations made on 5-day-old allatectomized males had suggested more pronounced effects on bands 3 and 4.

As expected, the depletion of most of the proteins of the ARG occurs in parallel with the formation of the spermatophore. Bands 1, 3, and 4 correspond to proteins of the utriculi (Feliubadaló et al., 1996). They are depleted during spermatophore formation, which suggests that they participate in this process. Conversely, band 2 corresponds to proteins of the uricose glands (Feliubadaló et al., 1996), and they are effectively depleted only after spermatophore transfer. This agrees with our observations (Feliubadaló and Bellés, unpublished data) indicating that the secretions of the uricose glands participate in sealing the freshly transferred spermatophore in the genital atrium. New accumulation of proteins in the ARG began just after mating, and 48 h later total contents were about 65% of those corresponding to 10-day-old virgin males, or equivalent to those measured on day 3–4 of adult life (Piulachs et al., 1992).

Concerning JH during and after mating, the results showed that rates of synthesis rarely exceeded 1 pmol/pair CA per h, which is usual in males of this species (Piulachs et al., 1992). The increase in biosynthetic rates observed after mating parallels the increase in total protein contents in the ARG, which were measured simultaneously. This suggests that increasing rates of JH synthesis are necessary to stimulate the progressive accumulation of proteins in the ARG, as occurs after the imaginal moult (Piulachs et al., 1992). What is intriguing is the significant decrease of JH synthesis observed during the formation of the spermatophore. Indeed, a plausible functional explanation for this is not obvious. However, it is worth noting that during the first cycle of maturation protein accumulation in the ARG stabilizes when the rate of JH biosynthesis is high (Piulachs et al., 1992), which suggests that high titres of JH might inhibit further protein synthesis in the ARG. Following this reasoning, the decrease of JH synthesis observed during the formation of the spermatophore would be necessary to start a new cycle of ARG maturation.

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