Experientia 36 (1980), Birkhäuser Verlag, Basel (Schweiz)

The transfer of juvenile hormone from male to female during mating in the Cecropia silkmoth[1]

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Summary. The juvenile hormone (JH) stored in the accessory sex glands (ASG) of adult male *Hyalophora cecropia* (L.) originates both from sequestration of circulating hormone and from JH synthesized de novo in the ASG from JH acid taken up from the hemolymph. The secretions present in the lumina of the ASG contain most of the accumulated JH. During mating, endogenous JH, labeled biosynthetically via injected [^{3}H-methyl]-methionine, is transferred along with the other seminal material to the bursa copulatrix of the female. The physiological significance of the JH transfer remains unknown.

The storage of large quantities of juvenile hormone (JH) in adult male Cecropia silkmoths[4] represents an atypical endocrine phenomenon which is known to occur in only 2 other closely related saturniids[5,6]. This phenomenon is based on the ability of the male accessory sex glands (ASG) to act as a repository for JH[7]. These glands contain a JH acid methyltransferase which facilitates the transfer of the methyl group of S-adenosyl methionine to a JH acid, thus forming the respective JH[8-11]. In addition to this process, the ASG are also able to take up JH intact. Once in the ASG, most of the JH stored is found in association with the secretions contained in the lumina of the glands[9]. Since the ASG contribute material to the formation of the spermatophore[12,13], we investigated the possibility of JH transfer to the female during copulation.

Cecropia were obtained from commercial suppliers as diapausing pupae and kept at 4 °C for a minimum of 90 days. Adult development was initiated by exposing the pupae to 27 °C, 70–80% relative humidity and 16–8 h light-dark cycle. [^{3}H-methyl]-methionine (3.7 Ci/mmole) was purchased from Schwarz/Mann. Ether was anhydrous, analytical reagent grade (Mallinckrodt).

Freshly eclosed adult male Cecropia were injected with [^{3}H-methyl]-methionine in Weevers' saline[14] (1 µCi/µl). After 24 h the radiolabeled males were placed with untreated freshly eclosed females. Adult Cecropia mate about 30 min before lights on, continue throughout the light period, and separate shortly after the lights go off again. Mating of all pairs occurred during the following dark phase and the day of copulation is referred to as day 0. The copulation of pair 1 was disturbed prior to the next dark phase and both animals were sacrificed, removing the male ASG and the female bursa copulatrix (BC) separately for processing. Pairs 2 and 3 were allowed to complete copulation; the males were sacrificed at the beginning of day 1 after copulation while the females were sacrificed on day 3 after copulation. The ASG and BC were processed for JH identification by extraction with ether/ethanol (6:1), TLC on silica gel HF_{254} (0.25 mm, methanol washed and activated) with a hexanes/ethyl acetate/acetic acid (70:25:5) solvent system, and high pressure liquid chromatography on µ Porasil with a hexanes/ethyl acetate/2-propanol (96.48:3.5:0.02) solvent system. A mixture of cold JH-I and JH-II was added to the ether/ethanol extracts as an internal standard.

As is evident in the table, JH accumulated in the ASG of adult males is transferred to the BC during copulation. When interrupted during mating, all of the labeled JH was found in the BC (pair 1). When the male was sacrificed after copulation, a small amount of radiolabeled JH was detected in the ASG (pair 2, pair 3), which suggests some post-coital accumulation of JH. It is also interesting to note that even 2 days after mating radiolabeled JH was present in the BC.

JH-I and JH-II in male accessory sex glands (ASG) and in the bursa copulatrix (BC) after copulation. Both juvenile hormones were labelled biosynthetically in the male before copulation

Mating Pair	Organ preparation Type of organ	Time after copulation (days)	JH-I (dpm)	JH-II (dpm)
1	ASG	Copulation interrupted	nil[a]	nil[a]
	BC	Copulation interrupted	9600	1600
2	ASG	0 days	2300	200
	BC	2 days	850	nil[b]
3	ASG	0 days	1000	nil[b]
	BC	2 days	10,100	2500
Control	ASG	Unmated male 2 days old	16,400	1800

[a] nil ≤ 60 dpm total between 2 adjacent fractions; [b] nil ≤ 600 dpm total between 2 adjacent fractions.

The transfer of JH from the male to the female Cecropia during mating is similar in many respects to the transfer of cantharidin between the males and females of *Lytta vesicatoria* (Spanish flies). The adult female *L. vesicatoria* is unable to produce any cantharidin but is known to contain this compound[16]. During copulation the cantharidin in the ASG of the male is transferred with the seminal materials to the female[15]. The function of the transferred JH remains unknown. Allatectomy clearly demonstrates that JH plays no role in reproductive processes of the Cecropia silkmoth[17,18].

1 Research was supported by a grant from the National Science Foundation (PCM72-01892) and Organized Research Funds from Texas A&M University.
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