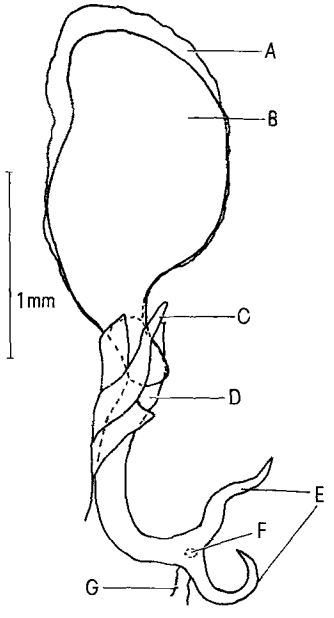
The Disruption of Neuro-Endocrine Function and Reproduction in the Mediterranean Flour Moth, *Ephestia kühniella* (Lepidoptera: Phycitidae) by a Chemosterilant, Hexamethylmelamine (HEMEL)

In a continuous effort to find relatively suitable chemosterilants for insect pests a significant level of mating aberrations have been observed in *Diparopsis castanea, Trichoplusia ni* and *Spodoptera littoralis* after treatment[1-3]. In the flour moth, HEMEL, when injected into adult males, induced some of them to undergo 2 mating aberrations (Table I), besides inducing significant reduction in egg hatchability. The 2 aberrations induced are sterile mating and permanent copulation. The former resulted in the female laying few or no eggs even though a spermatophore was found in the bursa copulatrix on dissection. This condition was similarly obtained when males were reared at high temperature, and was probably due to shortage of sperm[4]. During permanent copulation, the male which soon dies is normally dragged around by the female. This mating disfunction is caused by the inability of the male to completely introduce the spermatophore into the bursa copulatrix of the female during the process of spermatophore transfer. 14 pairs of insects in permanent copulation were examined, and the horns of the spermatophore were not dislodged from the horns of the ductus ejaculatorius (Figure). Further dissection of the bursa copulatrix revealed no blockage due to the presence of other spermatophores. Histological and histochemical examinations of the male reproductive organs generally did not reveal significant changes after HEMEL-treatment (unpublish data). However, even under normal conditions a few males were also found to undergo permanent copulation in the stock cultures. A further important finding was that injection of 16.7 µg HEMEL per male, induced spontaneous formation of spermatophore sac (without undergoing mating) in a significant number of control males but not in decapitated males. This suggested that the brain and the neuroendocrine complex may be involved in mediating the effects of HEMEL.



Position of spermatophore when permanent copulation occurs. A, bursa copulatrix (female); B, spermatophore sac; C, male clasper; D, adaegus enclosing spermatophore column; E, ductus ejaculatorius horns enclosing the horns of spermatophore; F, spermatophore opening; G, bulbus ejaculatorius.

Virgin male flour moths which emerged within 1 h of each other were injected either with 16.7 µg HEMEL or glass distilled water (controls). The heads were quickly severed, fixed and cross-sectioned serially at 6 µm, 3 days post treatment. The neuro-endocrine complexes from HEMEL-treated and control males were analyzed by staining the sections simultaneously with resorcin fuchsin under identical conditions[5]. No significant changes were observed in the corpora cardiaca and allata of the treated moths compared with the controls. However, the neurosecretory cells (A) in the medial group of the pars intercerebralis contained more abundant purple stained material in the treated insects than in the controls.

In another experiment, virgin males of *E. kühniella* were similarly treated as above except that no counterstaining was performed. The neurosecretory A-cells were scanned individually under a Vickers M85 microdensitometer in every section in which they appeared. The medial neurosecretory A-cells from HEMEL-treated males and their respective controls were scanned alternately in a day's operation. Table II shows that the stainable neurosecretory material in the A-cells was much higher in the HEMEL-treated insects than in the controls. However, a statistical analysis is not valid in this instance since a linear relationship between the optical absorbance and the integrated optical density could not be established. However, it is clear from this experiment that HEMEL treatment induced accumulation of neurosecretory materials either from increased synthesis or decreased release or both these processes in the A-cells.

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Table I. Mating aberrations induced by HEMEL

	Dose	No. of pairs	Control mating (%)	Permanent copulation of mated (%)	Sterile mating (%)[a]
Control	0.5 µl	16	93.7	0	0
HEMEL	10.1 µg	15	85.1	41.7	16.7
	16.7 µg	15	92.2	30.8	46.2

[a] Sterile mating in which mated female, as confirmed by the presence of spermatophore, laid few or no eggs.

Table II. Microdensitometric comparison of the neurosecretory materials in adult male *E. kühniella*

	No. of brain	No. of sections scanned	Integrated optical density[a]	
			Average	± S.E.
Control	5	53	4625.8	271.1
HEMEL	5	70	5323.3	216.7

[a] Measurements were done with reference to background at a wavelength of 580 nm, over circular area of 113 μm^2 under ×1,000 magnification in triplicates.

Table III. Cellular and nuclear volumes of neurosecretory A-cells and their nuclear-cytoplasmic ratio

	Control ± S.E.	HEMEL ± S.E.	Comparison of means
No. of brains	5	5	
No. measured	40	69	
Cell volume (μm^3)	618.2 ± 45.0	753.1 ± 44.6	$0.01 > P > 0.001$
Nuclear volume (μm^3)	99.4 ± 9.3	132.6 ± 8.6	$0.05 > P > 0.02$
Nuclear cytoplasmic ratio	0.205 ± 0.018	0.248 ± 0.020	$0.01 > P > 0.001$

The volumes of the A-cells and their nuclei were estimated from 2 radii perpendicular to each other obtained from camera lucida drawings based on the assumption that the cells are spheroids. The results indicated that HEMEL treatment had induced increased cellular activities of the A-cells, shown by the larger cellular and nuclear volumes compared with the controls (Table III). Further, the nuclear cytoplasmic ratio in the A-cells of treated males is also higher than the controls. These suggest that the accumulation of neurosecretory materials after treatment was largely due to increased synthesis relative to release.

The above experiments showed that the mating aberrations in *E. kühniella* induced by HEMEL are probably the result of a disruption in the normal functioning of the neurosecretory A-cells. However, a direct relationship between the function of the neurosecretory A-cells and normal spermatophore formation and transference has yet to be established. The induction of permanent copulation by disrupting neuro-endocrine function in an insect pest by chemosterilants is a special bonus when used in the sterile male release technique for the suppression of pests. The phenomenon of permanent copulation results in a plugging up of the female reproductive organs, hence few or no eggs are laid. This is particularly useful in the control of polyandrous pest such as *Ephestia*, since theoretically 1 male can completely suppress the reproductive potentialities of 1 female. The suppression of the female reproductive potentialities of polyandrous pest by conventional sterile male release would require a much higher ratio of sterile males to females than a method depending upon the phenomenon of permanent copulation as induced by neuro-endocrine manipulation. In addition permanent copulation also limits the movement of the insects and thus may further reduce the population by exposing them to predators.

We must differentiate clearly between the possible use of sterilants which induced permanent copulation and those which suppress reproductive activities through disrupting neuro-endocrine function. In the latter, mating is greatly reduced, as shown in a Tenebrionid beetle tranquilized by reserpine, as a result of accumulation of neuro-secretory material[6]. In the former case, the normal mating activities should be maintained or increased as compared with the controls.

Zusammenfassung. Injektion von Hexamethylamin in Männchen der Mehlmotte *Ephestia kühniella* bewirkt, dass sterile Spermatophoren übertragen werden oder dass die Kopula nicht mehr gelöst werden kann. Die Behandlung bewirkt auch eine Hyperfunktion der neurosekretorischen, medianen A-Zellen des Gehirns, die möglicherweise als Primäreffekt der Befruchtungsstörung vorausgeht.

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