The paraldehyde fuchsin positive material in corpora cardiaca of *Pimpla turionellae* L. (Hymenoptera:Ichneumonidae) during egg maturation

Aydın Özlük[1*] and Nursel Gül[2]

[1]*Hitit University , Faculty of Science and Art, Dept. of Biology, Çorum, Turkey*
[2]*Ankara University, Faculty of Science, Department of Biology, Ankara, Turkey*

Abstract

The purpose of this study is to examine the effects of the neurosecretory material in corpus cardiacum (CC) on the reproduction of endoparasitic *Pimpla turionellae* L. For this purpose, the egg maturation and amount of the neurosecretory material in corpus cardiacum of the insects were examined by the serial cross sections. Egg maturation was determined by measuring the terminal oocyte length in the serial cross sections of the ovariole. The egg growth which has observed on the 3rd day, reached the maximum on 15th day and after that day the egg laying was observed. The sections of insect brains were stained by paraldehyde fuchsin. The amount of the paraldehyde fuchsin positive neurosecretory (PF^+NS) material in CC was at a certain level in the first day of egg development. While terminal oocyte was reaching the maximum length, the amount of the PF^+NS material in CC decreased to the minimum level. During the egg laying phase, the amount of the PF^+NS material in CC reached the maximum level. These observations give us the idea that the neurosecretory material in corpus cardiacum of this insect may be related to the egg development.

Keywords: *Pimpla turionellae,* Corpora cardiaca, Egg maturation, Neurosecretory material, Paraldehyde fuchsin.
[*]**Corresponding address:** Aydın Özlük **(**E-mail: aydinozluk@hitit.edu.tr)
(Received: 02.06.2010 Accepted:03.09.2010)

Pimpla turionellae L.'nin (Hymenoptera:Ichneumonidae) yumurta gelişimi sırasında Korpora Kardiaka'sındaki paraldehit fuksin pozitif materyal

Özet

Bu çalışmanın amacı, bir dış parazit olan *Pimpla turionellae*'nin korpus kardiacum(CC)'undaki nörosekresyon maddesinin üreme üzerine olan etkisini incelemektir. Bu amaçla, seri kesitlerde, böceğin yumurta gelişimi ve korpus kardiacum'undaki nörosekresyon maddesi gözlenmiştir.Böceğin beyin kesitleri paraldehit fuksin ile boyanmıştır. Yumurta gelişimi, yumurtalık kesitlerindeki terminal oosit boyu ölçülerek belirlenmiştir. 3. günde gözlenen yumurta büyümesi, 15. günde en yüksek seviyesine ulaşmış ve bu günden sonra yumurta bırakma gözlenmiştir. CC'deki paraldehit fuksin pozitif (PF^+ NS) maddenin miktarı, yumurta gelişiminin ilk günlerinde belirli bir seviyedeydi. Terminal oositler en uzun boylarına ulaşırken, CC'deki PF^+ NS maddenin seviyesi en aza indi. Yumurta bırakma evresinde ise bu seviye, en yüksek değerine ulaştı.
Bu gözlemler bize, bu böceğin korpora kardiaka'sındaki nörosekresyon maddesinin yumurta gelişimi ile ilişkisi olabileceği fikrini vermiştir.

Anahtar Kelimeler: *Pimpla turionellae*, Korpora kardiaca, Yumurta gelişimi, Nörosekresyon maddesi, Paraldehit fuksin.

Introduction

In insects, the median neurosecretory cells of the protocerebrum produce cytoplasmic inclusions that stain with paraldehyde-fuchsin. Formerly, it was specified that the brain cells which are stained purple by paraldehyde fuchsin techniques include neurosecretory material (Ewen 1962). There are many studies on these neurosecretory cells with stained purple by paraldehyde fuchsin. (Siew 1965; Adams 1976; Juberthie and Caussanel 1980; Ulrich et al. 1985; Park and Ramaswamy 1998, Toyoda et al. 1999). These neurosecretory cells of the brain were shown as the control center of reproduction on *Calliphora erythrocephala* (Lea and Thomsen 1969), *Aedes aegypti* (Meola and Lea 1971) *Musca domestica* (Adams et al. 1975), *Locusta migratoria* (Ulrich et al. 1985), and were searched concerning egg maturation on *Lepismodes inquilinus* (Rohdendorf and Watson 1969), *Musca domestica* (Adams 1976), *Labidura riparia* (Juberthie and Caussanel 1980) and *Lymantria dispar* (Leob and Hayes 1980). The fact that the neurosecretory material which is produced in the median neurosecretory cells of the brain reaches to the corpora allata by passing through the corpora cardiaca via the nervi corporis cardiac, is well known. In CC of some insects, the neurosecretory material which is stainable with paraldehyde fuchsin positively, was presented (Meola et al. 1970; Khan et al. 1984; Toyoda et al. 1999). In insects, corpora allata and corpora cardiaca are known to be involved in metabolic activities (Kim et al. 2004; King et al. 2005; Tombes and Smith 2005).

As seen above, the relationship between the neuroendocrine system of the insects belonging to different orders and their reproduction activities were studied and various results were obtained. Previously the head endocrine system of *P.turionellae* had been given histologically and morphologically (Özlük 1991), but the physiological relationships between the endocrine system and the reproduction activity had not been mentioned. On the insects merged to Hymenopter order, it is rare to encounter studies that show the correlation between the brain neurosecretory cells and the reproduction. Especially, there is no study about the reproduction period which is related to the correlation between the neurosecretory material in CC and the egg development of *P. turionellae.*

This study observed and examined the role of the neurosecretory material CC in the egg development of the endoparasitic *P. turionella*. The study would seek to throw light on the relationship between the neurosecretory material in corpus cardiacum and reproduction in *P. turionellae.*

Material and Methods

Experimental Animals

Individuals of female *P. turionellae* were reared under laboratory conditions between the years 2003-2005 in the Insect Culture Laboratuary at Ankara University, Faculty of Science, Department of Biology. The female experimental animals were obtained from these stock culture. The continuity of the stock culture was supplied from the greater wax moth, *Galleria mellonellae*, reared on the semi-synthetic diet (Bronskill 1961). All *P .turionellae* females were kept at a temperature of 25 ± 2° C with relative humidity of 75 ± 5 percent in a 12 : 12 (L : D) photoperiod. They were fed with cotton pieces absorbed with 50% honey solution and a pupa of *G. mellonellae* was given to each insect every two days in order to satisfy their host haemolymph needs. All the experimental virgin females were reared without males to eliminate possible effects of mating (Acle' et al. 1990). They were selected on the 0, 3, 6, 9, 12, 15, 18, 21, 24 and 27th days, after adult emergence. All experimental females were selected in the same body size to eliminate the difference in terminal oocyte length (Briegel 1990).

Staining of the Brain and Ovaries

All females were decapitated at the same time ($14.^{00}$-$17.^{00}$) to eliminate the possible diurnal changes in the neurosecretory system (De Wilde 1964; Engelmann 1968). The dissected brain of *P. turionellae* was fixed in aqueous Bouin's solution for 12-18 hours, sectioned serially at 7 milimicron thickness after being embedded in paraffin. These sections of the brain were stained with paraldehyde fuchsin after potassium permanganate oxidation (Panov 1980). The rest of the body of the decapitated animals was dropped into isotonic insect saline and was left for no longer than 3 hrs. The ovaries were extracted and dissected, for microscopic calculation, under a binocular microscope and were fixed in Bouin's fixative (4 hrs). Dissected ovaries were sectioned serially at 10 milimicron. The sections were stained with Ehrlich's haemotoxylin and eosin (Pearse 1961).

Measurements

The lengths of the terminal oocyte were measured with an ocular micrometer. The developmental stages of the oocyte growth were recorded as the length of the biggest terminal oocyte in ovariole (Pratt and Davey 1972). All measurements were made in the largest area in the cross sections of the terminal oocytes. Only one biggest terminal oocyte length from each of at least 10 animals in each studied day was measured and their averages were recorded. The measurements in the terminal oocytes were done by Fisher's (1948) method of significance control test ($P < 0.05$) between two means.

In order to point out the changes in amount of the neurosecretory material in corpus cardiacum on the experiment days, the paraldehyde fuchsin positive granules in CC were counted. The paraldehyde fuchsin positive granules in CC were numbered 0 to 5 increasing according to its staining intensity (Adams et al.1975). An average value for each day investigated was obtained by dividing the sum of the staining intensity of CC into the number of CC of each the day. These calculations were done for each insect separately.

Results

Terminal Oocyte Growth

The larger oocytes in any ovariole represent a longitudinally arranged series in which the terminal oocyte nearest the base of the ovariole is the oldest and largest oocyte representing successively earlier stages in the oocyte maturation (Pratt and Davey 1972). In all *P. turionellae* females, there is no differentiation in the ovariole on the day of emergence (Table 1). The terminal oocytes continue to increase in length between the 3rd day and 15th day.

Table 1. The terminal oocyte lengths of *P. turionellae* on different days of egg maturation.

Age at Dissection (Day)	Insect Number of Terminal Oocyte Length Measured	Terminal Oocyte*	
		Numbers	Length(µm) ± SD
0	15	-**	-**
3	13	13	445.38 ± 17.95a***
6	10	10	621.67 ± 28.16a
9	14	14	801.03 ± 19.63bc
12	14	14	935.58 ± 17.65b
15	10	10	1092.63 ± 23.10b
18	10	10	777.34 ± 39.46a
21	10	10	470.96 ± 40.25ac
24	11	11	736.39 ±31.13a
27	10	10	837.82 ± 33.14bc

* Only one terminal oocyte was measured in each female

** There is no terminal oocyte in ovariole on the day of emergence

*** Means compared vertically. Means not followed by the same superscript are significantly different from each other at $P < 0,05$ by Fisher's T test.

Between the 12th day and 15th day, the length of the terminal oocyte reaches a maximum level which is called egg maturation stage. Between the days 15th and 21th, the length of the terminal oocyte decreases to a minimum. These days, on which the terminal oocyte length decreased to minimum, are called the egg laying stage.

The Paraldehyde Fuchsin Positive Material in Corpus Cardiacum

The corpora cardiaca are elongated paired bodies laying behind of the brain, dorsal to the hypocerebral ganglion. They are intimately associated with the walls of the aorta, anterior to the point where the vessel bends downwards beneath the brain, following the course of the oesophagus. The anatomy of the corpora cardiaca and their relation with the aorta of *P.turionellae* has been described by Özlük (1991).

The amount of the paraldehyde fuchsin positive material in corpus cardiacum had different levels during the egg development period of the females of *P.turionellae* (Table 2).

Table 2. The average amount of the paraldehyde fuchsin positive material in corpus cardiacum of *P. turionellae* on different days of egg maturation.

Age at Dissection (Day)	Insect Number	Amount of PF^+NS material in CC	
		Total	Average*
0	22	59	2.7
3	22	66	3.0
6	8	7	0.9
9	40	12	0.3
12	24	0	0.0
15	18	16	0.9
18	20	200	5.0
21	12	48	4.0
24	28	8	0.3
27	22	44	2.0

* The average amount of the paraldehyde fuchsin positive (PF^+NS) material in corpus cardiacum (CC) was found by dividing the total intensity of neurosecretory material to the insect number.

The amount of the paraldehyde fuchsin positive material in corpus cardiacum in the newly emerged insects was on a limited scale and the level was not low. This intensity level slightly increased on the first days depending on the ooctye maturation and showed fluctuation on the following days. The paraldehyde fuchsin positive neurosecretory material intensity which decreased to the minimum level before the first egg laying phase days, reached a high intensity on the first egg laying days. After the first egg laying days the amount of neurosecretory material decreased considerably. This fluctuation of the amount of the paraldehyde fuchsin positive material in corpus cardiacum during egg maturation can be mentioned as there is a correlation between the paraldehyde fuchsin positive material in corpus cardiacum and egg maturation on the reproduction period in this insect.

Discussion

A brain cell has been considered as neurosecretory if stained purple with paraldehyde fuchsin, and only the purple condition has been considered indicative of the presence of neurosecretory material and has been called paraldehyde fuchsin positive (Ewen 1962). The fact that the median neurosecretory cells of insects includes PF^+NS material was shown by the studies done by means of light and electron microscope. (Gilbert and Dia 1993; King et al. 2005; Tombes and Smith 2005; Bonetti et al. 2006). In the meantime the PF^+NS material exists also in the CC of female of *P.turionellae* and was marked in the serial sections which were obtained from the head of the insect (Özlük 1991). In many earlier studies, the neurosecretory hormones had already been chosen to show their cyclic activity (Leob and Hayes 1980; Greenplate et al. 1985; Huerta and Martinez 2008) correlated with the oocyte maturation (Lea and Thomsen 1969; Bell and Barth 1971; Meola and Lea 1971; Tabakoğlu 1988; Toyoda et al. 1999). In *P. turionellae* females, the day of emergence was characterized by the lack of differentiation in the ovariole. On this day, the amount of the PF^+NS material in CC was at a certain level. During the first days of the terminal oocyte growth, a permanent increase or decrease was not observed in the amount of the PF^+NS material in CC. After the first days of egg maturation, this level showed fluctuation. But, especially the level which has the minimum value just before the first egg laying days (on 12^{th} day), increased considerably on the first egg laying days (between 18^{th} day and 21^{st} day). This reduction which occurred just before terminal oocytes reached the maximum length was assessed as there has been no more need to

hormones since the egg maturation was completed. It has been known that the PF^+NS material in the brain passes to corpora allata throughout corpora cardiaca. Thus, the changes in the amount of PF^+NS material in CC can be explained with the changes in the intensity of PF^+NS material in the brain. Adams et al. (1968) specified that the neurosecretory material which is secreted from median neurosecretory cells, provides oocyte maturing. Adams specified with another researcher group in 1975 that there is a feedback mechanism between the brain cells of *M. domestica* and ovariums. Adams, (1976) also specified that the oocytes which complete the maturation, produce a hormone which blocks the secretion activity of the brain that is called oostatic hormone. This specification of Adams can be explained by the reduction of PF^+NS material in CC of *P. turionellae* on the 12^{th} day. The striking increase in PF^+NS material in CC of *P. turionellae* on the first egg laying days also can be explained with the suggestion of Siew (1965) which shows that egg laying activity requires a high hormone level. When injected the brain extract which is obtained from the egg laying females of *Gryllus bimaculatus* to young mature females, Sefiani (1987) observed that they also show egg laying activity and abdominal contractions start. Thus, it was recommended that more neurohormones are required for egg laying and this neurohormone causes abdominal contractions and egg laying movements. The amount of PF^+NS material in CC of *P. turionellae* decreased after the egg laying phase. As a matter of fact the neurosecretory material intensity which has a considerably high level during the egg laying phase, decreased after the egg laying activity completed in *L.riparia* (Juberthie and Caussanel 1980), in 9-24 hours after the egg laying activity in *Galeruca tanaceti* (Siew 1965), and in 12-36 hours in *M. domestica* (Adams et al. 1975).

The results obtained from the present study show that the amount of PF^+NS material in corpus cardiacum of *P. turionellae* changes during the reproductive activity. The egg laying and the egg maturation phases of *P. turionellae* are correlated with the neurosecretory material in corpora cardiaca which can be specified from these results. An estimation is also given of the storage and release of a neurosecretory hormone that is essential for egg laying and egg maturation in *P. turionellae*.

References

Acle' D., Brookes, V.J., Pratt G.E. and Rene' F. R. (1990) Activity of the corpora allata of adult female *Leucophaea maderae*: Effects of mating and feding. *Archives of Insect Biochemistry and Physiology,* 14(3): 121-129.

Adams T.S., Hintz M.M. and Pomonis J.G. (1968) Oostatic Hormone Production in Houseflies, *Musca domestica*, with Developing Ovaries. *Journal of Insect Physiology,* 14: 983-993.

Adams T.S., Grugel S., Ittycherian P.I., Olstad G. and Caldwell J.M. (1975) Interactions of the ring gland, ovaries, and juvenile hormone with brain neurosecretory cells in *Musca domestica*. *Journal of Insect Physiology*, 21: 1027-1043.

Adams T.S. (1976) The ovaries, ring gland, after neurosecretion during the second gonotrophic cycle in the housefly**,** *Musca domestica*. *General and Comperative Endocrinology*, 30: 69-76.

Bell W.J., and Barth R.H. (1971) Inititation of yolk deposition by juvenile hormone. *Nature, New Biology*, 230, 220-221.

Bonetti A.M., Siquieroli A.C., Santana F.A., Vieira C.U., Bezerra M., Pereira G.B., Simões Z.L. and Cruz-Landim C. (2006) Effect of juvenile hormone III on the ultrastructure of the corpora allata in *Melipona quadrifasciata* (Hymenoptera, Apidae, Meliponını). *Brazilian Journal of Morphological Science*, 23(3-4): 393-400.

Briegel, H. (1990) Metabolic relationship between female body size, reserves and fecundity of *Aedes aegypti*. *Journal of Insect Physiology*, 36(3): 165-172.

Bronskill J.F. (1961) A cage to simplify the rearing of the greater wax moth, *Galleria mellonella* (Pyralidae). *Journal of Lepidopteran Society*, 102-104.

De Wilde J. (1964) *Reproduction-Endocrine Control*. Physiology of Insecta (Ed. by. Rockstein, M). 1: 59-88.

Engelmann F. (1968) Endocrine control of reproduction in insects. *Annual Review of Entomology*, 13: 1-26.

Ewen A.B. (1962) An improved aldehyde fuchsin staining technique for neurosecretory products in insects. *Transmission American Microscopy Society*, 81: 94-96.

Fisher R.A. (1948) Statical tables for biological agricultural and medical research. *3. ed. Oliver and Boyd. Ltd. Edinburg.*

Gilbert I.L. and Dia J. (1993) An ultrastructural and developmental analysis of the corpus allatum of juvenile hormone deficient mutants of *Drosophila melanogaster***.** *Roux's Archieves of Developmental Biology*, 202: 85-94.

Greenplate J.T., Glaser R.L. and Hagedorn H.H. (1985) The role of factors from the head in the regulation of egg development in the mosquito *Aedes aegypti*. *Journal of Insect Physiology*. 31: 325-329.

Huerta C. and Martinez M.I. (2008) Morphological changes in reproductive organs and neuroendocrine centers related to nesting, mating, and larvicide behavior in *Eurysternus mexicanus* harold (Scarabaeinae: Eurysternini). *The Coleopterists Bulletin*, 62(1): 123-132.

Juberthie C. and Caussanel C. (1980) Release of Brain Neurosecretory Products from the Neurohaemal Part of the Aorta During Egg-Laying and Egg-Care in *Labidura riparia*, (Insecta: Dermaptera). *Journal of Insect Physiology*, 26: 427-429.

Khan M.A., Romberg-Privee H.M. and Schooneveld H. (1984) Innervation of the corpus allatum in the Colorado Potato Beetle as revealed by retrograde diffusion with horseradish peroxidase. *General and Comperative Endocrinology*, 55: 66-73.

Kim S.K., Seung K. and Rulifson E.J. (2004) Conserved mechanisms of glucose sensing and regulation by *Drosophila* corpora cardiaca cells. *Nature*, 16.431: 316-320.

King R.C., Aggarwal S.K. and Bodenstein D. (2005) The comparative submicroscopic cytology of the corpus allatum-corpus cardiacum complex of wild type and *fes* adult female *Drosophila melanogaster*. *Journal of Experimental Zoology,* 161(2): 151-175.

Lea A.O. and Thomsen E. (1969) Size idependent secretion by the corpus ailatum of *Calliphora erythrocephala*. *Journal of Insect Physiology*, 15: 477-482.

Leob M.J. and Hayes D.K. (1980) Neurosecretion during diapause and diapause development in brains of mature embryos of the gypsy moth, *Laymantria dispar*. *Annual Entomological Society of American*, 73: 432-436.

Meola R. and Lea A.O. (1971) Independence of paraldehyde-fuchsin staining of the corpus cardiacci and the presence of the neurosecretory hormone required for egg development in mosquito. *General and Comperative Endocrinology*, 16: 105-111.

Meola A. R., Lea A.O. and Meola R. (1970) Corpus cardiacum: Induced Fluctuation in Paraldehyde-Fuchsin Material in Aedes sollicitans *Transactions of the American Microscopical Society* . Vol. 89, No. 3: 418-423.

Özlük A. (1991) The cerebral neurosecretory system and the diversity of the neurosecretory cell types in *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae). *Communications.* Fac. Sic. Univ. Ank. Ser*: C.*, 9: 33-47.

Panov A.A. (1980) Demonstration of neurosecretory cells in the insect central nervous system. In "Neuroanatomical Techniques*" Ed by NJ Strausfeld, TA Miller, Springer-Verlag*, New York, 25–50.

Park Y.I. and Ramaswamy S.B. (1998) Role of Brain, Ventral Nerve Cord, and Corpora Cardiaca-Corpora Allata Complex in the

Reproductive Behavior of Female *Tobacco budworm* (Lepidoptera: Noctuidae). *Annual Entomological Society of America*. 91(3): 329-334.

Pratt G.E. and Davey K.G. (1972) The corpus allatum and oogenesis in *Rhodnius prolixus* (STAL.) I. The effects of allatectomy. *Journal of Experimental Biology*. 56: 215-222.

Pearse A.G.E. (1961) Histochemistry, *Theoretical and Applied*, 2 nd. ed. J. and A. Curchii Ltd. London.

Rohdendorf E.B. and Watson J.A.L. (1969) The Control of Reproductive Cycles in the Female Firebrat, *Lepismodes inquilinus*. *Journal of Insect Physiology*, 2085-2101.

Sefiani M (1987) Regulation of Egg Laying and in Vitro Oviducal Contractions in *Gryllus bimaculatus. Journal of Insect Physiology*, 3(4): 215-222.

Siew Y.S. (1965) The Endocrine Control of Adult Reproductive Diapause in the Chrysomelid Beetle, *Galeruca tanaceti* (L.), III. *Journal of Insect Physiology*, 2: 973-981.

Tabakoğlu A. (1988) Neurosecretory cell types and "C - cysteine incorporation in adult female *Calliphora vicina*. ROB. - DESV. (ERYTH-ROCEPHALA MEIG.) *Istanbul Universitesi Fen Fakültesi Biyoloji Dergisi*, 53: 45-64.

Tombes A. and Smith D.S. (2005) Ultrastructural studies on the corpora cardiaca-allata complex of the adult alfalfa weevil, *Hypera postica. Journal of Morphology*, 132(2): 137-147.

Toyoda I., Numata H. and Sakiko S. (1999) Role of the Median Neurosecretory Cells in the Ovarian Development of the Blow Fly *Protophormia terraenovae. Zoological Science*, 16(2): 187-199.

Ulrich G.M., Schlagintwelt B., Eder J. and Rembold H. (1985) Elimination of the allatotrophic activity in locust by microsurgical and immunological methods: Evidence for humoral control of the corpora allata, haemolymph proteins, and ovary development. *General and Comperative Endocrinology*, 59: 120-129.