□보 문□

韓應昆誌 30(1):29~36(1991)
Korean J. Appl. Entomol.

Studies on the Light Organ of the Firefly, *Luciola lateralis* Motschulsky

애반딧불이의 발광기관 구조

Dae Woo Lee and Kyung Saeng Boo[1]
이 대 우·부 경 생[1]

ABSTRACT Studies were carried out to investigate structural characteristics of the larval and adult light organs of *Luciola lateralis* Mot. and to observe the relation between the light organ and nerve. The larval light organs, (LLO) existed at a paired dorsal lateral positions of the 8th abdominal segment. The organ was spherical or sub-spherical in shape. There were many vacuoles around the LLO. As larva grew, the number of vacuoles increased. LLO had the muscles in its interior part and their role seemed to fix the LLO position in space. Also, there were the tracheae and tracheoles in LLO. The adult light organs (ALO) were at the ventral portions of the 5th and 6th abdominal segments in the male, but only in the 5th abdominal segment in the female. ALO had two functional layers, i. e., photocyte and dorsal layer. Tracheal end organs existed in both layers but their arrangements were irregular. Rod-shaped photocytes and spherical photocytes were observed in the ALO of male and female, but the rod-shaped types were rarely found in the female. In the ALO of the 5th abdominal segment, two paired peripheral nerves were originated from the anterior part of the last abdominal compound ganglion. A pair of peripheral nerves were originated from the posterior part of the compound ganglion to innervate the 6th abdominal segment ALO. And LLO was innervated by a paired peripheral nerves from the last abdominal ganglion.

KEY WORDS *Luciola lateralis*, adult light organ, larval light organ, bioluminescent organ

초 록 애반딧불이 (*Luciola lateralis* Mot.) 성충과 유충 발광기관의 현미경 관찰결과와 발광기관과 신경계의 관계는 다음과 같다.
유충의 발광기관은 복부 8번째 마디 좌우 등쪽 측면에 한 쌍이 존재하였으며 복부 8번째 신경절로부터 나온 한쌍의 말초신경에 지배받는다. 발광기관의 모양은 구형 혹은 구형과 유사한 형태이며 발광기관 주변에는 과립들이 있었다. 이러한 과립들은 유충령기가 높아갈수록 많아져 용화하기 위하여 지상에 상륙한 유충의 경우, 하나의 층을 형성하고 있는 것처럼 보였다. 그리고 발광기관에는 근육이 존재하며 기관과 기관소지도 발견되었다. 성충의 발광기관은 수컷의 경우 복부 5, 6째 마디에 있으며 암컷의 경우는 5째마디에만 존재했다. 성충의 발광기관은 발광세포층(약 70㎛의 두께)과 반사층(약 40㎛의 두께)으로 구성되어 있었다. 그리고 유충의 발광기관에서는 볼 수 없었던 氣管枝小器官(tracheal end organ)이 존재하였다. 복부 5째 마디의 발광기관은 복합신경절 (유충의 6째, 7째, 8째 복부마디의 신경절들이 융합된) 앞쪽에서 나온 두 쌍의 말초신경에 의해 지배되며, 복부 6째 마디(수컷)의 발광기관은 복합신경절의 뒤쪽에서 나온 한쌍의 말초신경에 지배받는다.

검 색 어 애반딧불이, 성충발광기관, 유충발광기관

In fireflies, all stages of life cycle can be luminescent. However, true light organs are usually observed only in the larval and adult stage, but they differ in structure and luminous pattern. The larval

1 Dept. of Agricultural Biology, College of Agriculture, Seoul Natl. University, Suwon 441-744, Korea(서울대학교 농과대학 농생물학과)

light organ degenerates while the adult light organ differenciates and receives new neural afferents during the pupal stage. Of course the larval light organ excision does not interfere with formation of the adult light organ (Harvey & Hall 1929). The firefly light organ, therefore, is particularly valuable since development of the new effector organ during pupal stage can be studied, especially on its nerve connection, without disturbing the nervous system artificially (Strause et al. 1981). Also firefly bioluminescence is utilized for ATP assay, biomass assay of microorganisms, luminescence immunoassay and so forth.

In Korea 7 species of the fireflies were reported (Kang et al. 1974). But investigations about the fireflies are almost nil. Because the fireflies, except for *Luciola lateralis* Mot, inhabit mountains and valleys, their collection is not easy. Since *L. lateralis* inhabits rice-fields and its flight activity is not active, their collection is very easy. However, population of *L. lateralis* has been in decline due to wide use of agro-chemicals and environmental contamination.

The present study was carried out to investigate structural chracteristics of the larval and adult light organs in *L. Lateralis* and to observe the relation between the light organ and nerves for a future study for a control mechanism of the luminous reaction.

MATERIALS AND METHODS

Adult fireflies (*Luciola lateralis*) used in this study were collected at Seolchun, Chonbuk and Mt. Weorak, Chungbuk. Climbing larvae and pupae were obtained from reared larvae in laboratory (water temp., 22 ± 1℃; photoperiod, 16L : 8D). These larvae were initially collected at rice-paddy fields of Seolchun, Chonbuk. Each larval instar was distinguished by length of prothoracic cuticle plate (Yuma 1986).

Central nervous system observations and gross innervation of the adult and larval light organ

Samples at various developmental stages were dissected under a stereomicroscope. The viscera were carefully removed to expose the ganglia. After dissection, the ventral nerve cord and its segmental nerves were stained with 0.3% methylene blue solution for 1-2 seconds. And the surplus methylene blue was washed by tap water.

Paraffin embedding method for light microscopy

Samples were fixed with the Bouin's fixative for 4 hours, dehydrated with ethanol and xylene, and embedded with paraffin. Sections (10㎛) were stained with the Mallory's triple staining solution.

Resin embedding method for light microscopy

The light organs of the fireflies were cut out in cold (4℃) 2.5% glutaraldelyde solution in 0.1M phosphate buffer (pH 7.2) and fixed in the same, fresh fixative for 2-4 hrs. The fixative also contained 0.2M sucrose for a proper osmolarity. Specimens were rinsed in the 0.1M phosphate buffer. Afterwards, they were postfixed with buffered 1% osmium tetroxide for 10-12hrs., dehydrated in acetone and embedded in Agar 100 resin. Sections (1㎛) were stained with the solution of 1% toluidine blue or methylene blue in 1% borax and mounted with Canada balsam.

RESULTS

Light organs of larvae

The larval light organ(LLO) of *L. lateralis* located in a paired dorsal lateral positions of the 8th abdominal segment. LLO was spherical or sub-spherical in form with the diameter of 96㎛ (4th instar larva),

133 ㎛ (5th), 151 ㎛ (6th) and 201 ㎛ (climbing larva). LLO did not have the reflector layer. There were many vacuoles around the LLO. The more larva grew, the more number of vacuoles were observed (Figs. 1, 2, 3). In LLO of climbing larva the number of those vacuoles remarkably increased. They seemed to form a layer (Fig. 4).

As in LLO of other species, tracheae and tracheoles were distributed in LLO (Fig. 6). But this firefly LLO had the muscles specially in interior part (Figs. 7, 8).

Light organs of adults

The adult light organs (ALO) were in the ventral portions of 5th and 6th abdominal segments in males (Fig. 10) and only in 5th abdominal segment in females.

The length of ALO in the 5th segment was 2.1 ㎜ (male) and 2.3 ㎜ (female). The depth was about 0.5 ㎜. The length and depth of 6th ALO (male) were 1.4 ㎜ and 0.4 ㎜. Unlike LLO, ALO had two functional layers. The photocyte layer (depth: about 70 ㎛) was directly contacting the integument. The dorsal layer (depth: about 40 ㎛) surrounded the photocyte layer and had the compacted urate granules. In LLO, nerve did not make the complex with the tracheoles but directly connected with photocytes. But ALO made the tracheal end organs (the complex of nerves and tracheoles). Therefore, nerves and tracheoles connected the photocytes indirectly. The tracheal end organs existed in the photocyte layer (Fig. 12), but some of them started to show up in the dorsal layer, just above the photocyte layer. A tracheal end organ was surrounded by several photocytes (Fig. 13). Embedded paqttern of tracheal end organs at the photocyte layer was irregular.

Photocytes were diverse in their form and staining reaction. In their form they were distinguished into two types, rod-shaped (20-60 ㎛ long and 8-20 ㎛ wide) (Fig. 11a) and spherical (10-30 ㎛ in diameter) (Fig. 11b) cells. Both cell types were observed in the ALO of male and female, but female rarely had the rod-shaped cells. Also there were heavily stained cells and lightly stained cells when stained with methylene blue or toluidine blue.

The relation of nervous system and firefly light organ

Bioluminescence organs of both larva and adult were innervated by nerves from the last abdominal ganglion. The ganglion was 8th abdominal one in larva, but 5th one in the adult, a compound one formed by fusion of 3 last ganglia in the larvae.

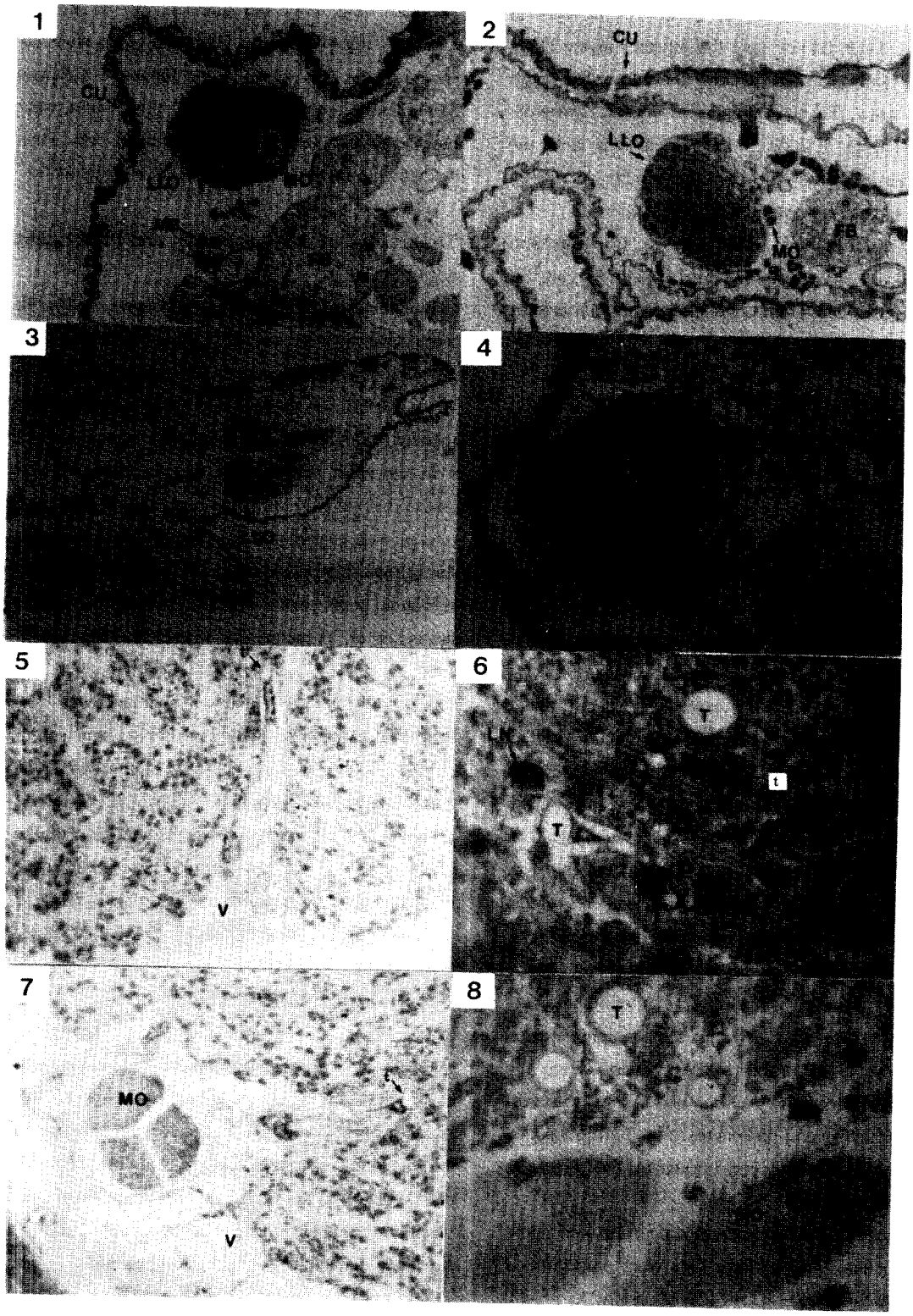
In the adult light organs, the origin of the peripheral nerves were different from LLO's. That was related with changes of the central nervous system during metamorphosis (Fig. 14). For a detailed observation, pupal period was divided into three stages depending on its morphological chracteristics (Table 1).

ALO of the 5th abdominal segment was controlled by two paired peripheral nerves which were originated from the anterior part of the compound ganglion. The 6th abdominal ALO was controlled by a paired peripheral nerves which came out of the posterior part of the compound ganglion. And LLO was controlled by a paired nerves which were distributed from the last ganglion (Fig. 15).

DISCUSSION

Firefly light organs show an astonishing diversity of structure and can be classified in a number of arbitrary ways. Buck (1984) divided the light organ into 6 types. According to Buck, ALO of *L. lateralis* can be placed in the type 5. In this type the tracheal supply arboresces among the photocytes. That is similar to the light organ of *Luciola cruciata*.

As in other cases, ALO of *L. lateralis* consists of


1
CU
LLO
2
CU
LLO
FB
MO
3
4
5
V
6
T
LL
t
T
7
MO
V
8
T

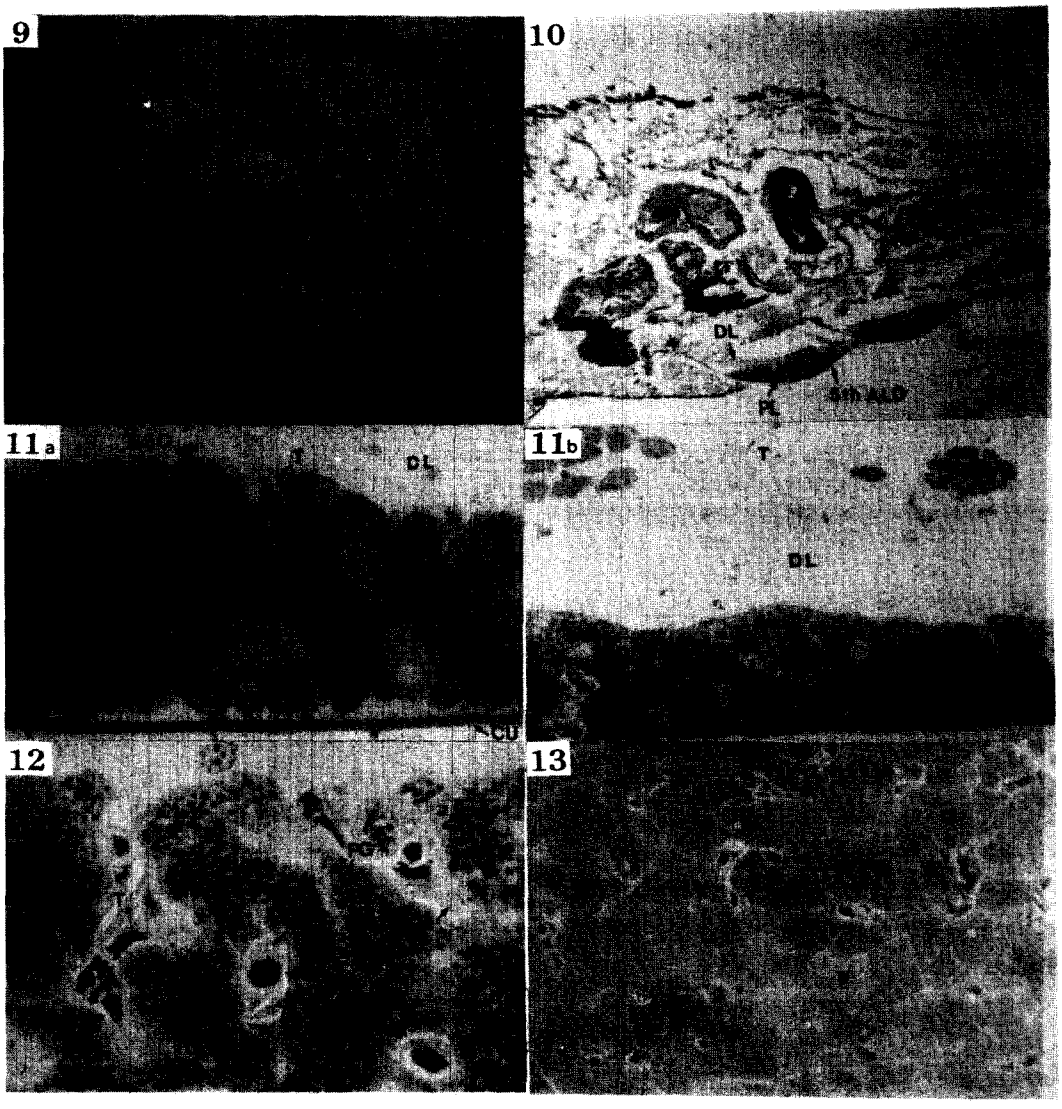


Fig. 1. Part of the 8th abdominal segment from the 4th instar larva of *L. lateralis* (cross section, ×200).
Fig. 2. Part of the 8th abdominal segment from the 5th instar larva of *L. lateralis* (longitudinal section, ×200).
Fig. 3. Part of the 8th abdominal segment from the 6th instar larva of *L. lateralis* (longitudinal section, ×100).
Fig. 4. Part of the 8th abdominal segment from the climbing larva of *L. lateralis* (cross section, ×200).
Fig. 5. An outer edge of the light organ from the 4th instar larva of *L. lateralis* (cross section, ×1000).
Fig. 6. Tracheae of the light organ from *L. lateralis* 6th instar larva (longitudinal section, ×1000).
Fig. 7. Cross section of a muscle holding the larval light organ *L. lateralis* (see fig. 1) (×1000).
Fig. 8. Longitudinal section of a muscle holding the larval light organ of *L. lateralis* (see fig. 3) (×1000).
Fig. 9. Cross section of the 5th abodominal segment from *L. Lateralis* adult female (×40).
Fig. 10. Longitudinal section of the 5th and 6th abdominal segment from *L. lateralis* adult male (×40).
Fig. 11a. Adult male photocytes are composed of rod- or spherical-shaped cells and also of heavily and lightly stained cells (×400).
Fig. 11b. Most of adult female photocytes are spherical in shape and heavily stained (×200).
Fig. 12-13. Tracheal end organs formed in the photocyte layer of the adult light organ (Fig. 12; cross section, ×1000; Fig. 13; horizontal section, ×400).

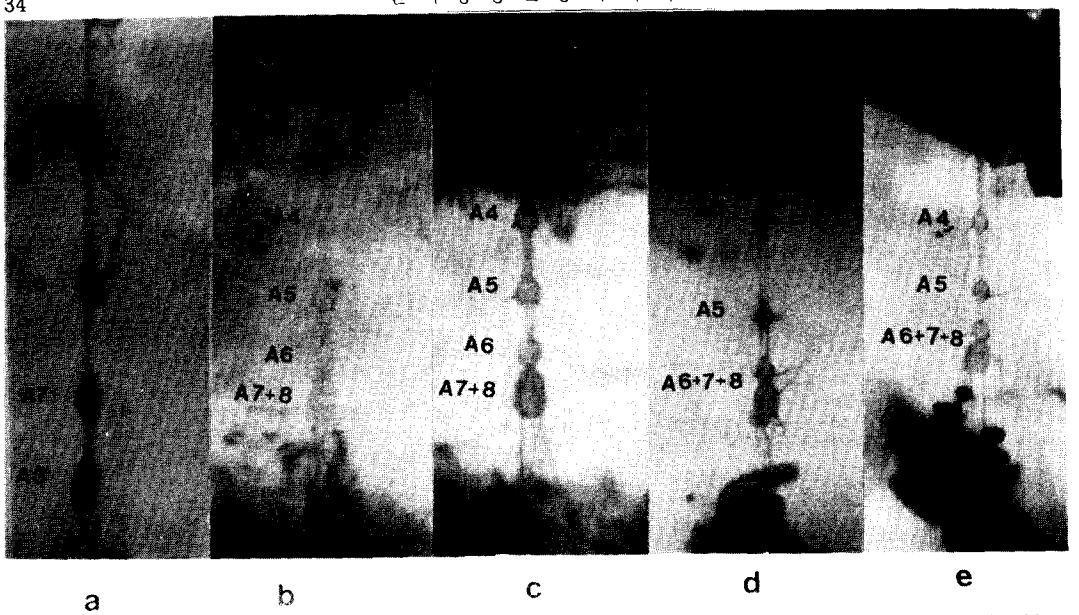


Fig. 14. Metamorphic changes of the posterior abdominal ganglia of *L. lateralis*; (a) larva, (b) early pupa, (c) midstage pupa, (d) late pupa and (e) adult. Numbers refer to abdominal ganglia, with 6, 7 and 8 forming the adult's terminal ganglion.

the dorsal layer and the photocyte layer and has tracheal trunks and tracheal end organs. Trachea, surrounded by tracheal epithelial cells, run through both layer. In the photocyte layer, however, instead of forming a cylinderical enlargenent it is enlarged like tree roots as in *Luciola cruciata* (Buck 1984). The tracheal end cells are filled with relatively long mitochondria and spherical mitochondria as shown by Beams and Anderson (1955).

The photocytes of *L. lateralis* adult are diverse in their form and staining reaction. There are many questions about such phenomena. The difference in form could be related to the different luminescence pattern of male and female, since rod-shaped cells are abundant in the male but only a few in the female. The difference of heavily and lightly stained cells seems to be caused by the density of photocyte granules. This could he due to different location of granules with in photocytes. But for resolution of the reasons related to such differences more investigation must be executed.

The photocytes surround the tracheal end organs. They are associated with tracheal end organs in a rosette fashion (Fig. 15). But their arrangement is irregular unlike *Pteroptryx* sp. (Peterson & Buck 1968). A photocyte contains a number of granules, called photocyte granules. The shape of photocyte granule is spherical and its size is variable. Between photocyte granules, mitochondria exist. The photocyte granules are not observed in the differential zone that is the part of photocyte, adjacent to the tracheal end organ. Rod- and spherical-shaped mitochondria are observed in that zone. Except for the shape of photocytes and tracheal emedding fasion, this firefly ALO is similar to those of other fireflies (Beams & Anderson 1955, Kluss 1958, Smith 1963, Peterson & Buck 1968, Hanna et al. 1976).

ALO is innervated by the last ganglion which is a compound structure due to a fusion of 6th, 7th and 8th abdominal ganglia of larva (Fig. 20). The compound ganglion is located through 4th and 5th abdominal segments. In *Photuris* sp. the ventral nerve cord from the last two abdominal ganglia innervates

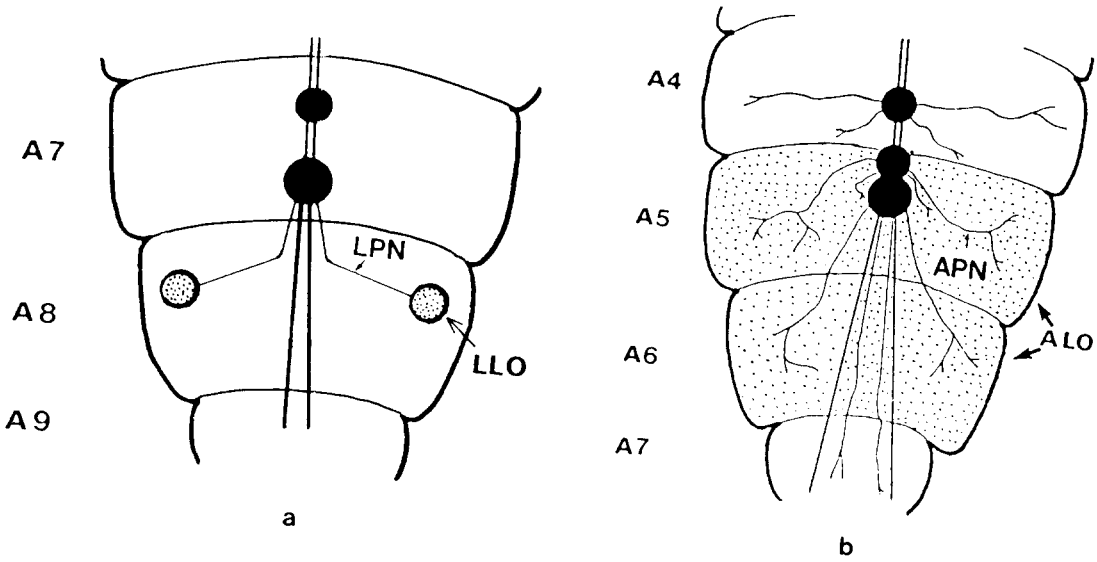


Fig. 15. Longitudinal diagram of the posterior part of ventral nervous system innervating the light organ. a) larva b) adult

Table. 1. Duration (day) and morphological and biological characteristics of pupal stages

Stage	Duration	Characteristics
Early pupa	4 ± 0.5	Whole pupa is colorless except for late appearing pigmentation at the periphery of the compound eyes. A paired LLOs glow.
Midstage pupa	2 ± 0.2	Compound eyes protrude and darkly pigmented. Antennae, mouth parts & legs are lightly pigmented. A paired LLOs glow.
Late pupa	1.7 ± 0.4	Dark pigmentation of the wing and abdominal segments. Usually ALOs appear in the 5th and 6th abdominal segments. Both LLOs and ALOs are capable of light emission.
Total	7.8 ± 0.8	

its ALO. Both of these ganglia are situated in the 6th abdominal segment (Hanson 1962, Carlson 1969). In *Photuris pennsylvanica* the last abdominal ganglion of mid-pupa is a compound one due to a fusion of the 7th and 8th abdominal ganglia of larva (Strause et al. 1981). Such difference is related with changing process of ganglia and ALO position. ALO position *L. lateralis* is in the 5th and 6th segments but that of *Photuris* ALO position is in the 6th and 7th segments.

Many people have been interested in the larval emission but there are few studies about structure of LLO. It has been reported in only two studies (Peterson 1970, Oertel et al. 1975). The LLO of *L. lateralis* does not have the reflector layer and its shape is spherical or sphere-like. Several fat bodies

surround the light organ. LLO is not in contact with the integument directly and some space exists between the cuticle and the light organ. There are many vacuoles around the LLO. The number of vacuoles increase remarkably in LLO of climbing larva. LLO of climbing larva does not differ from the others except for increasing its LLO size and number of vacuoles. But climbing larva still emited relatively intensive light. Therefore it seems that the vacuoles are related to the light intensity of LLO (for instance, role of reflector layer). But more study is needed for its resolution. Also, LLO has the muscles that are not reported in LLOs of other species. As the LLO is always at the same position it seems that muscles fix LLO position in the hemocoel (Figs. 7, 8). The overall shape of LLO is smilar to the lateral luminous organ of *Phrixothrix* sp. (Buck 1948). And tracheal end organs are not found in the LLO of this species. Tracheae are observed mainly at the proximal part of LLO. But tracheoles are distributed randomly. It is supposed that the nerves exist at LLO but more investigation is needed for this point.

REFERENCES CITED

Beams, H. W. & E. Anderson. 1955. Light and electron microscope studies on the light organ of the firefly, *Photinus pyralis*. Biol. Bull. 109 : 375~393.

Buck, J. B. 1948. The anatomy and physiology of the light organ in fireflies. Ann. N. Y. Acad. Sci 49 : 397 ~482.

Carlson, A. D. 1969. Neural control of firefly luminescence. Adv. Insect Physiol. 6: 51~96.

Hanna, C. H., T. A. Hopkins & J. B. Buck. 1976. Peroxisomes of the firefly lantern. J. Ultrastr. Res. 57: 150~162.

Hanson, F. E., Jr. 1962. Observation on the gross innervation of the firefly light organ. J. Insect Physiol. 8: 105~111.

Harvey, E. N. & R. T. Hall. 1929. Will the adult firefly luminescence if its laval organs are entirely removed? Science 69 : 253~254 (Indirectly cited from Bioluminescence in Action. 1978. ed. by Herring, P. J. pp331~366. Academic Press, New York).

Kang, Y. S., C. H. Kim, H. S. Kim, Y. G. Pack, D. S. Lee, B. H. Won, B. S. Cho & K. S. Choi. 1974. Nomina Animalium Koreanorum. Vol. 2 Insecta. 334pp. Hyang Mun Sa. Seoul.

Kluss, B. C. 1958. Light and electron microscopic observation on the photogenic organ of the firefly, *Photuris pennsylvanica*, with special references to the innervation. J. Morph. 103: 159~186.

Oertel, D., K. A. Linberg & J. F. Case. 1975. Ultrastructure of the larval firefly light organ as related to control of light emission. Cell Tiss. Res. 164 : 27~44. (Indirectly cited from Bioluminescence in Action. 1978. ed. by Herring, P. J. pp331~366. Academic Press. New York).

Peterson, M. K. 1970. The fine structure of the larval firefly light organ. J. Morph. 131 : 103~116.

Peterson, M. K. & J. B. Buck. 1968. Light organ fine structure in certain Asiatic fireflies. Biol. Bull. 135: 335~348.

Smith, D. S. 1963. The organization and innervation of the luminescent organ in a firefly, *Photuris pennsylvanica*(Coleoptera). J. Cell Biol. 16 : 323~359.

Strause, L. G. & J. F. Case 1981. Neuropharmacological studies on firefly light organs during metamorphosis. J. Insect Physiol. 27 : 5~15.

Yuma, M. 1986. Growth and size variations in the larvae of *Luciola cruciata* (Coleoptera: Lampyridae) in relation to the egg size. Physiol. Ecol. Japan 23: 45~78.

(Received Aug. 6, 1990)