Proc. Arthropod. Embryol. Soc. Jpn. 41, 47–53 (2006)

ISSN 1341–1527

Embryonic Development of the Firefly *Pyrocoelia rufa* Olivier (Insecta: Coleoptera, Lampyridae), with Special Reference to Its Hibernal Diapause

Yukimasa KOBAYASHI[1], Tomo WATANABE[2] and Hirobumi SUZUKI[3]

[1] *Department of Biological Science, Graduate School of Sciences and Engineering, Tokyo Metropolitan University, Minami-ohsawa 1–1, Hachioji, Tokyo 192–0397, Japan*
[2] *Futamatagawa 1–64–1–432, Asahi-ku, Yokohama, Kanagawa 241–0821, Japan*
[3] *Hino 5979–1, Hino, Tokyo 191–0012, Japan*
E-mail: kobayashi-yukimasa@c.metro-u.ac.jp (YK)

Abstract

The embryonic development of the firefly *Pyrocoelia rufa* was described with special emphasis on its hibernal diapause at the early embryonic stage. In Tsushima Isl., Japan, the females lay their eggs in October, and the eggs hibernate and hatch out in May of the next year; thus the egg period is about eight months. Under the rearing condition with the temperatures close to the outdoor ones, a small ball-shaped germ rudiment is formed by invagination of the germ disk into the yolk at about 5 days after oviposition. The germ rudiment then migrates near the center of the egg within several days, and the egg enters diapause at this stage. The egg resumes its development at about 165 days after oviposition and hatches out at about 230 days after oviposition; thus the egg diapause lasts about five month. The mode of embryogenesis of *P. rufa* as a whole, that is, the formation of the ball-shaped germ rudiment in the yolk and the submerged condition of the embryo, is fundamentally the same as that of other fireflies, although the mode is singular in insect embryogenesis. However, the occurrence of long diapause at the stage of the ball-shaped germ rudiment is reported here for the first time in Lampyridae.

Introduction

Pyrocoelia rufa Olivier belongs to a family of beetles known as the Lampyridae or fireflies, but its adult females are wingless. Although this species is widely distributed in northern China and the Korean Penisula, it inhabits only in Tsushima Island in Japan. In this island, the adults emerge in autumn (October), and females lay their eggs immediately after copulation. The egg period lasts about eight months, and consequently the larvae hatch out in the early summer (May) of the next year; thus, this species hibernates in the egg stage. Hibernation in the egg stage is exceptional in the life history of Lampyridae, only known in this species and its allied one, *Pyrocoelia atripennis* (Ohba, 1980). This peculiar life history thus arouses much interest in the subjects; whether the embryonic diapause occurs during the egg period, and if it occurs, at what stage the eggs hibernate. There is, however, no information as to the embryonic development of *P. rufa*. Then in this paper, we describe the outline of its embryogenesis, and report the occurrence of very long diapause in the early embryonic stage for the first time in Lampyridae.

Materials and Methods

Four females of *Pyrocoelia rufa* were captured at Tsushima Isl., Nagasaki Prefecture, Japan, at the beginning of October of 2006. The females deposited their eggs in masses on moist filter papers in Petri dishes in a laboratory in mid-October. Although the number of eggs per female was highly variable from 22 to 145, a total of about 300 eggs were obtained. The newly laid eggs were soon transferred to other Petri dishes lined with moistened filter paper and incubated at a constant temperature 20℃ during the first 30 days after oviposition. During the 31st to 230th days (until hatching), in order to bring this condition of incubation close to the change of temperatures in the habitat (Tsushima Isl.) of this species, temperatures for incubation were at first lowered and later elevated by stages as shown in Figure 1; the temperature during the 31st to 74th days was maintained at 15℃, during the 75th to 150th days at 4℃, during the 151st to 195th days at 10℃, and during the 196th to 230th days (until hatching) at 20℃. The eggs were fixed with hot alcoholic Bouin's fluid (about 55℃ for 30 min) every 12 h for the first 24 h after oviposition. During the second to 15th days they were fixed every 24 h. During the 31st to 165th days they were fixed every 15 days. During the 166th to 230th days (until hatching) they were fixed every 9 or 10 days.

For histological observation, after removing the chorion with fine needles, the eggs were dehydrated in an ethanol series, embedded in paraffin, sectioned at 5 or 7 μm, and stained with Delafield's hematoxylin and eosin. For observation of the whole embryos by light microscopy, the fixed eggs were stained with alcoholic borax carmine.

Observation

The newly laid eggs of *Pyrocoelia rufa* are pale yellow, nearly spherical and about 1. 8 mm in diameter. It is difficult to distinguish the anterior and posterior poles, or the polarity of the egg, because of its almost complete spherical shape. However, two minute, circules are observed on both sides of the egg surface, or on the chorion; one circle is about 70 μm in diameter, composed of about 10 brown spots, and the other is smaller than this and composed of three or four brown spots. One of these circles or both of them are supposed to be micropylar region(s), but we could not confirm their function.

The processes of maturation division, fertilization and cleavage were not observed in detail. During the first 48 h after oviposition, however, most of the cleavage nuclei reach the egg periphery, and a thin cellular blastoderm is formed on the egg surface. During the next 24 h, the blastoderm differentiates into two areas; the germ disk, or the future embryonic area, composed of thick columnar cells having one nucleus, and the extraembryonic one, or the future serosa, composed of large, cubic cells having two or four nuclei (Fig. 2A). The germ disk is circular and about 300 μm in diameter, and is always situated under or near one of the circular lines on the chorion. That is, in five of seven eggs observed, the germ disk was formed near the small circle on the chorion, while in other two eggs near or just beneath the large one. All marginal regions of the germ disk then flex, being accompanied by the extraembryonic area, to form an amnioserosal fold, whereas the central part of the germ disk becomes thick and then begins to sink into the yolk (Fig. 2B).

From 72 h to 96 h after oviposition, the amnioserosal fold extended from all margins of the germ disk eventually meets and fuses, whereas the germ disk forms itself into a ball-shaped germ rudiment having a minute lumen in its center (Fig. 2C). The extraembryonic area also meets and fuses to form a serosa covering the whole egg surface. On the other hand, the ball-shaped germ rudiment is separated from the developing serosa, and a small cavity, or the amniotic cavity of 70–90 μm in diameter, is formed in its center (Fig. 2D). In parallel with the formation of the germ

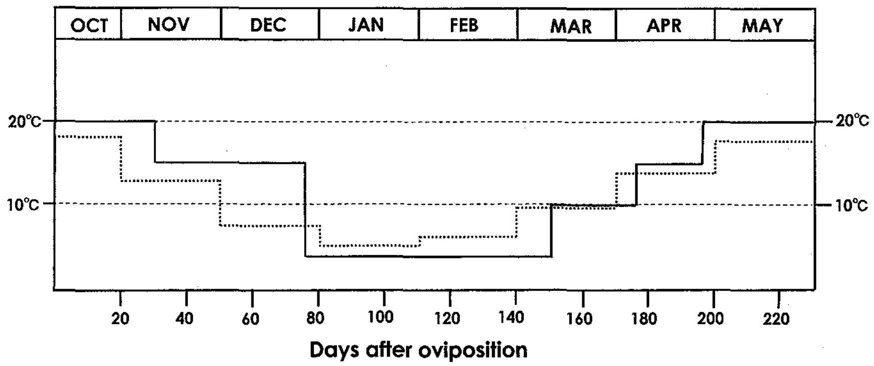

Fig. 1 Temperatures for incubation of eggs (solid line) and mean temperatures for a month at Izuhara in Tsushima Island (dotted line) during the egg period of *Pyrocoelia rufa*. Meterological data of this island were based on Chronological Scientific Tables 2005 edited by National Astronomical Observatory.

rudiment, yolk cleavage occurs, that is, the yolk mass becomes divided into numerous, nucleated, spherical portions or yolk cells, each of which is wrapped in a delicate membrane (Fig. 2C, D).

The germ rudiment then deeply sinks into the yolk and migrates near the center of the egg at about 11 days after oviposition (Fig. 2E). Under the rearing condition, the germ rudiment then stops its development and enters hibernal diapause until about 170 days after oviposition, with retaining its original ball-shape and size, about 220 μm in diameter (Fig. 2F).

At about 170 days after oviposition, the ball-shaped germ rudiment begins to elongate and becomes a comma-shaped one about 500 μm long; thus the egg resumes its development. Accompanying the elongation of the germ

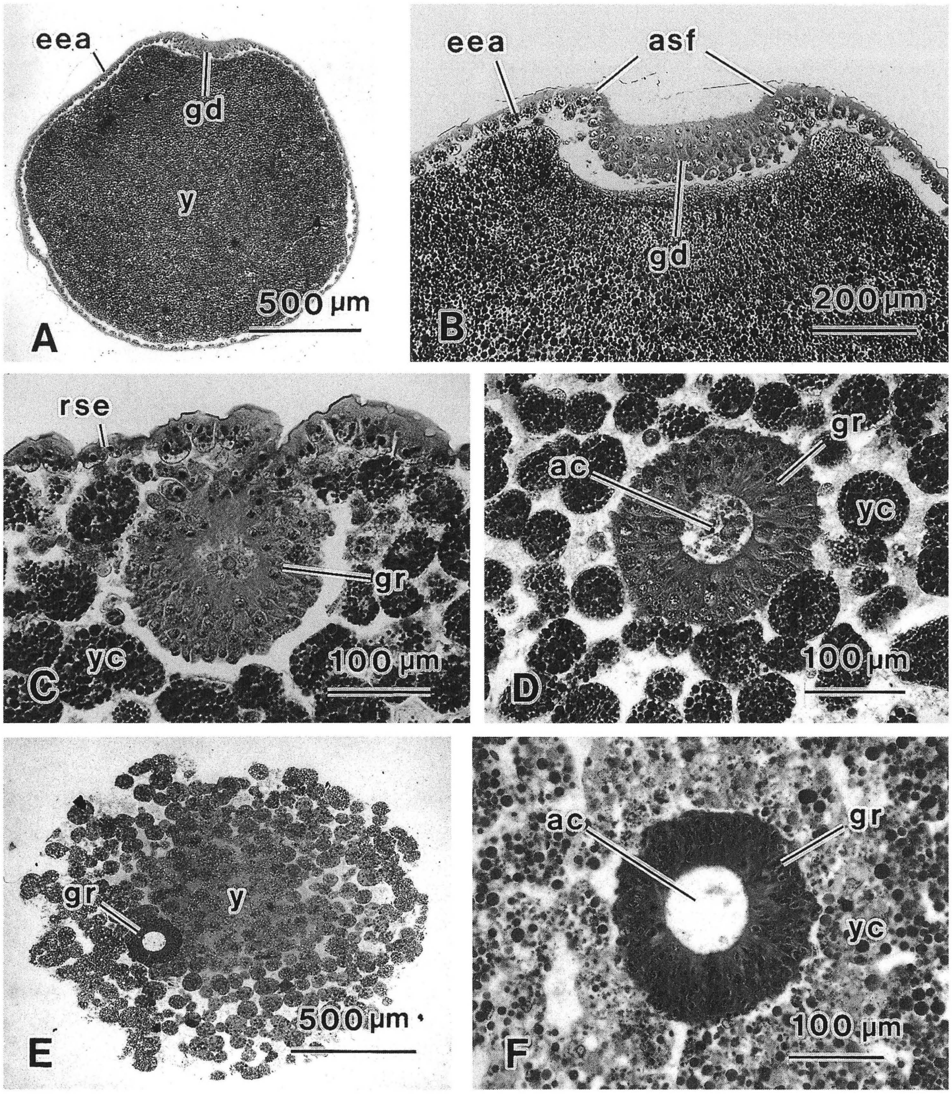

Fig. 2 Sections of *Pyrocoelia rufa* eggs. A. About 2-day egg showing the differentiation of germ disk and extraembryonic area. B. about 2-day egg showing the germ disk and amnioserosal fold. C. About 3-day egg showing the ball-shaped germ rudiment and rudimentary serosa. D. About 5-day egg showing the developing amniotic cavity in the germ rudiment. E. About 11-day egg showing the germ rudiment migrated near the center of the egg (serosa was removed). F. About 31-day egg showing the germ rudiment during diapause. ac: amniotic cavity, asf: amnioserosal fold, eea: extraembryonic area, gd: germ disk, gr: germ rudiment, rse: rudimentary serosa, y: yolk, yc: yolk cell.

rudiment, proliferation of cells, or the formation of the primitive groove, occurs along the midline of the concave side of the germ rudiment (Fig. 3A–D). This concave side is the embryo proper, and the other side is the rudimentary amnion. The primitive groove then becomes a sunken tube through the union of its lateral margins beneath the embryo. The tube flattens into a continuous two-layered sheet of cells forming the inner layer or mesoderm; thus the embryo becomes composed of the outer ectoderm and inner mesoderm.

At about 185 days after oviposition, the embryo becomes fully elongated and its segmentation is completed; that is, the embryo is now composed of the protocephalic region, four gnathal, three thoracic, and 10 abdominal segments. With the elongation of the embryo, the rudimentary amnion thins and becomes a definitive amnion. At about 195 day after oviposition, the rudimentary antennae appear as a pair of appendages on both sides of the protocephalic region and the rudimentary labrum is formed at the anterior end of the protocephalic region. Three pairs of gnathal appendages, *i. e.*, mandibular, maxillary, and labial ones, and thoracic appendages also appear in the gnathal and thoracic segments, respectively (Fig. 3E). In the first abdominal segment, the pleuropodia appear as a pair of dome-shaped projections. The embryo, however, is still completely immersed in the yolk at this stage. The embryo then further widens and becomes superficial in position from its anterior part, that is, the cephalic segments assume a superficial position first (Fig. 3F), and finally the whole embryo becomes observable from the outside. During this period the pleuropodia become large and finally develop into saucer-shaped structures. The embryo then accomplishes revolution at about 210 days after oviposition, and the definitive form of the first instar larva is established at about 225 days after oviposition.

Hatching occurs at about 230 days after oviposition.

Discussion

Mode of embryogenesis in Lampyridae

Embryological studies of Lampyridae have been restricted to the following five species; *Photuris pennsylvanica* (Williams, 1916; Hess, 1922), *Photinus consanguineus* (Williams, 1916), *Luciola cruciata* (Ando and Kobayashi, 1975; Kobayashi and Ando, 1985; Kobayashi, 1987), *L. lateralis* (Kobayashi and Ando, 1985), and *Hotaria palvula* (Kobayashi and Ando, 1985; Kobayashi, 1987). The first species belongs to the subfamily Photurinae, and the second to the subfamily Lampyrinae. The last three species belong to the other subfamily Luciolinae. In all these species, a ball-shaped germ rudiment is formed by invagination or ingrowth of the circular germ disk into the yolk irrespective of the different subfamilies they belong. In recent years, it was confirmed that a similar, spherical germ rudiment is also formed in a glowworm, *Rhagophthalmus ohbai*, which belongs to the family Rhagophthalmidae allied to the Lampyridae (Kobayashi *et al.*, 2001, 2002). In both of these families, the germ rudiment then becomes a long germ band or embryo in the yolk, and the submerged condition of the embryo persist until just before embryonic revolution. Therefore, this type of germ rudiment formation and the submerged condition of the embryo appear representative of the Lampyridae and its allies, and are singular in insect embryogenesis.

In *Pyrocoelia rufa*, which belongs to the subfamily Lampyrinae, the ball-shaped germ rudiment is also formed, and the embryo develops in the submerged condition in the yolk until just before revolution. Thus the mode of its embryogenesis as a whole is the same as that of the lampyrid and rhagophthalmid species mentioned above.

Hibernal diapause in the early embryonic stage of Pyrocoelia rufa

The only difference in embryogenesis between *Pyrocolelia rufa* and other lampyrids is a very prolonged stage of the ball-shaped germ rudiment lasting about five months in the former species. This stage most likely corresponds to a hibernal diapause stage, because the eggs experience the coldest season during this stage. As shown in Figure 4, however, under the rearing condition of this study, the ball-shaped germ rudiment was completed at about 5 days, and it migrated near the center of the egg by about 11 days. The germ rudiment then arrested its development, far before the rearing temperature was lowered to the level of 15℃ at 31 days. Therefore, it is assumed that the onset of diapause is independent of temperature, and the arrest of the development belongs to obligatory diapause, not facultative one. On the other hand, morphological changes of the germ rudiment, or the resumption of development, occurred at about 170 days after oviposition, or about 20 days after the temperature was raised to 10℃ from 4℃. Although we did not conduct a control experiment in which the eggs are kept at a constant temperature 20℃, it may fairly be presumed that the resumption of development, or the diapause termination, was induced through the

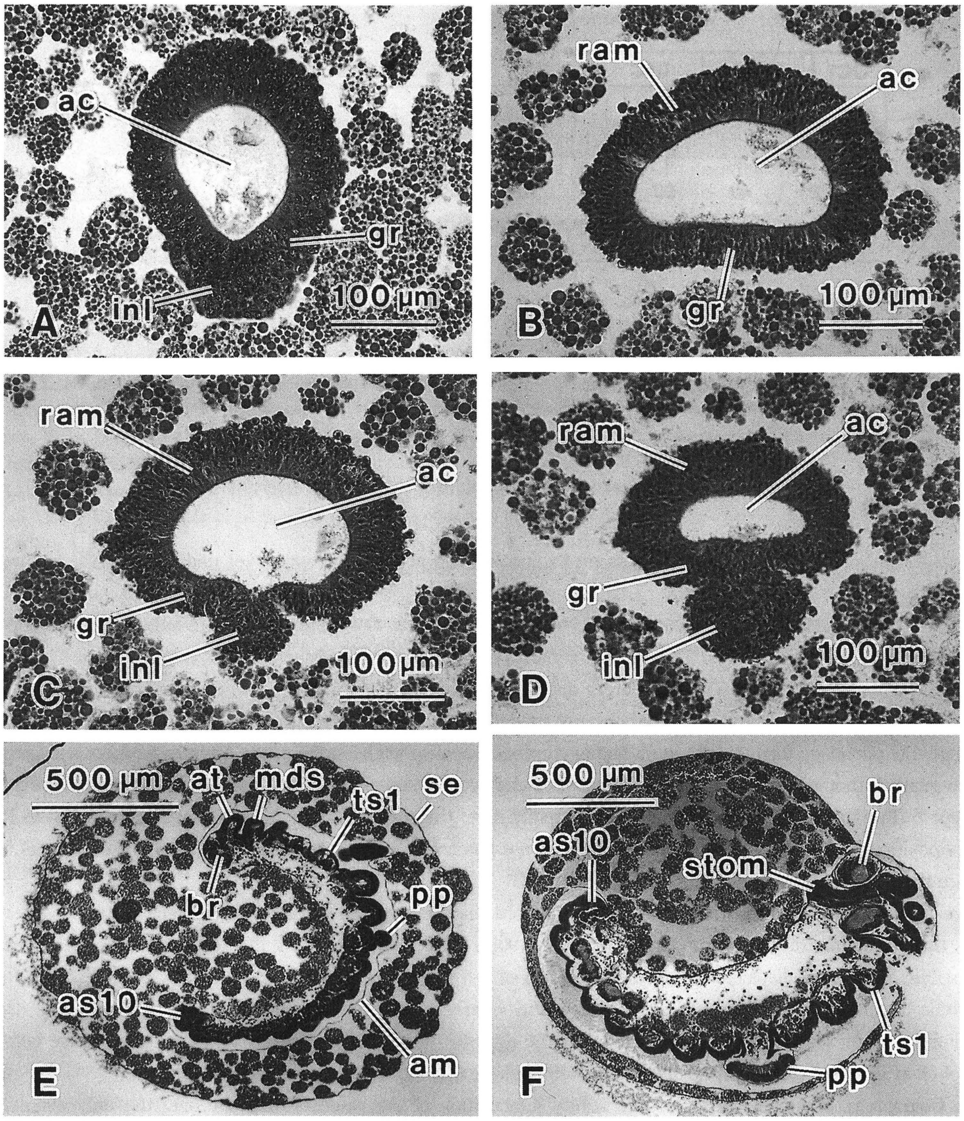

Fig. 3 Sections of *Pyrocoelia rufa* eggs. A. About 165-day egg showing the beginning of the inner layer formation in the germ rudiment. B–D. About 175-day egg showing the cross sections at the anterior one third (B), middle (C), and posterior one third (D) of the elongated germ rudiment of the same egg. E. Sagittal section of about 195-day egg showing the developing embryo. F. Sagittal section of about 205-day egg showing the embryo slightly before embryonic revolution. ac: amniotic cavity, am: amnion, as10: 10th abdominal segment, at: rudimentary antenna, br: brain, gr: germ rudiment, inl: inner layer, mds: mandibular segment, pp: pleuropodium, ram: rudimentary amnion, se: serosa, stom: stomodaeum, ts1: prothoracic segment.

preservation of eggs at a low temperature for a sufficiently lasting term, because it is generally accepted that a low temperature is effective for the termination of diapause in insects.

In many insects that hibernate in their egg stage, the arrest of development, or diapause, takes place at a different stage of development in different species. Umeya (1946, 1950) classified the egg diapause of insects, chiefly in Lepidoptera and Orthoptera, into five types according to the developmental stages of embryos in which diapause is observed morphologically. The five types are as follows.

Type 1 (Pyriform stage): The egg enters diapause at a very young and small embryonic stage known as the

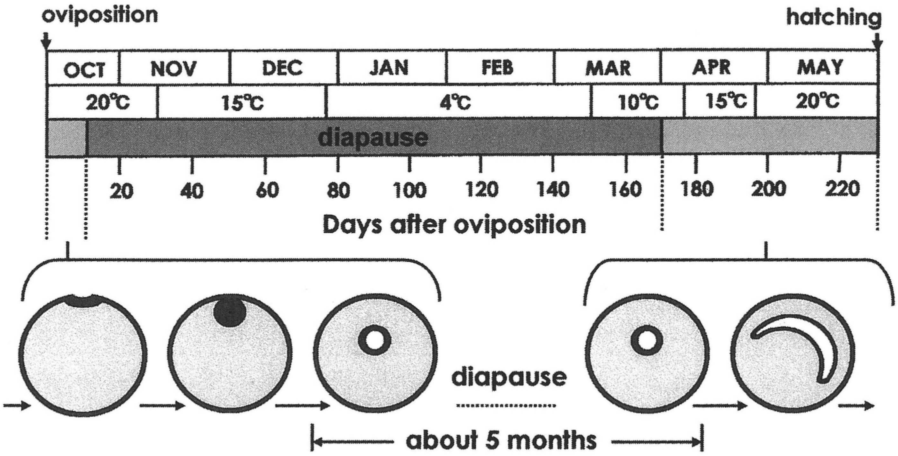

Fig. 4 Summary of embryonic development of *Pyrocoelia rufa* with emphasis on diapause.

"pyriform stage" in Lepidoptera in which its protocephalon and protocorm remain yet undifferentiated. In this stage the inner layer also is not yet formed.

Type 2 (Dumb-bell shaped stage): The egg enters diapause at the stage of a dumb-bell shaped embryo with enlarged cephalic and caudal lobes. The embryo consists of the ectoderm and the inner layer (mesoderm).

Type 3 (Elongation stage): The egg enters diapause at the stage of a highly elongated embryo. In this stage the embryo has a full set of segments, but without any appendages.

Type 4 (Appendage formation stage): The egg enters diapause at the stage of the embryo with appendages in the cephalo-gnathal, thoracic, and abdominal regions. The embryos of this stage do not yet undergo embryonic revolution.

Type 5 (Pre-larval stage): The egg develops rapidly up to the stage nearly of larval formation within 10 to 12 days after oviposition in summer. The egg, however, does not hatch out, unless exposed to the low temperature of 5–10℃ for more than two months.

According to Umeya and others, Lees (1955) stated that "no species are known in which diapause supervenes before the formation of the blastoderm." Masaki (1960), however, suggested that the three cricket species of *Dianemobius* may enter diapause before the formation of the germ band, and Tanigawa *et al.* (2000) reported that the embryonic development of *Dianemobius nigrofasciatus* is arrested at the blastoderm stage in its hibernal diapuase eggs. Furthermore, Hartman (1980) reported that the eggs of the crane fly *Tipula simplex* enter diapause before the onset of cleavage or at the fusion nucleus stage, although he did not show any histological figures.

In Coleoptera, however, we have only scanty information of the stage of diapause, and the only example was shown in the eggs of a chrysomelid beetle, *Atrachya menetriesi* (Miya, 1965). In this species, the egg enters diapause at the appendage formation stage, thus belongs to the type 4 by Umeya's classification.

In *P. rufa*, the egg enters diapause at the stage of the ball-shaped germ rudiment, or at the very early embryonic stage not only before the formation of the inner layer but also before the differentiation of the amnion and embryo proper. The type of egg diapause in this species, therefore, may be close to the type 1. In Umeya's type 1, however, it is assumed that the differentiation of the amnion from the pyriform embryo is completed, although he does not refer to this phenomenon. The ball-shaped germ rudiment of *P. rufa* thus can be regarded as a slightly younger stage than the pyriform stage with the completed amnion. Hence our observation probably affords one example of very early stages of diapause in insect embryogenesis.

The adults of *Pyrocoelia atripennis*, which is closely related to *P. rufa*, also emerge late in autumn in Yaeyama Islands, Okinawa, and their eggs hibernate. It might be an interesting subject to be resolved whether the eggs of *P. atripennis* enter diapause at an early embryonic stage as in the case of *P. rufa*.

Acknowledgments: We thank Dr. N. Ohba of the Yokosuka City Museum for his advice on the life history of *Pyrocoelia*. We are also indebted to Dr. S. Masaki of the Hirosaki University and Dr. H. Numata of the Osaka City

University for giving us valuable information on the development of *Dianemobius*.

References

Ando, H. and H. Kobayashi (1975) Description of early and middle developmental stages in embryos of the firefly, *Luciola cruciata* Motschlusky (Coleoptera: Lampyridae). *Bull. Sugadaira Biol. Lab., Tokyo Kyoiku Univ.*, **7**, 1–11.

Hartman, M.J. (1980) Embryonic diapause in *Tipula simplex* and the action of photoperiod in its termination (Diptera: Tipulidae). *Pan-Pacific Entomol.*, **56**, 207–212.

Hess, W.N. (1922) Origin and development of the light-organs of *Photurus pennsylvanica* De Geer. *J. Morphol.*, **36**, 245–277.

Kobayashi, H. (1987) Embryonic development of fireflies, *Luciola cruciata, L. lateralis* and *Hotaria parvula. Bull. Sugadaira Montane Res. Ctr. Univ. Tsukuba*, **8**, 141–153. (in Japanese with English summary).

Kobayashi, H. and H. Ando (1985) Early embryogenesis of fireflies, *Luciola cruciata, L. lateralis* and *Hotaria parvula* (Coleoptera, Lampyridae). *In* H. Ando and K. Miya (eds.), *Recent Advances in Insect Embryology in Japan*, pp. 157–169. Arthropodan Embryological Society of Japan, Nagano. (K.K. ISEBU, Tsukuba).

Kobayashi, Y., H. Suzuki and N. Ohba (2001) Formation of a spherical germ rudiment in the glow-worm, *Rhagophthalmus ohbai* Wittmer (Coleoptera: Rhagophthalmidae), and its phylogenetic implications. *Proc. Arthropod. Embryol. Soc. Jpn.*, **36**, 1–5.

Kobayashi, Y., H. Suzuki and N. Ohba (2002) Embryogenesis of the glowworm *Rhagophthalmus ohbai* Wittmer (Insecta: Coleoptera, Rhagophthalmidae), with emphasis on the germ rudiment formation. *J. Morphol.*, **253**, 1–9.

Lees, A.D. (1955) *The Physiology of Diapause in Arthropods*. Cambridge University Press, Cambridge.

Masaki, S. (1960) Thermal relations of diapause in the eggs of certain crickets (Orthoptera: Gryllidae). *Bull. Fac. Agr. Hirosaki Univ.*, **6**, 5–20.

Miya, K. (1965) The embryonic development of a chrysomelid beetle, *Atrachya menetriesi* Faldermann (Coleoptera). I. The stages of development and changes of external form. *J. Fac. Agr. Iwate Univ.*, **7**, 155–166.

Ohba, N. (1980) *Lychnuris* (= *Pyrocoelia*) *rufa* in Tsushima Island. *Bull. Yokosuka City Mus.*, **26**, 15–19. (in Japanese).

Tanigawa, N.A., H. Numata, S. Shiga and K. Yasuyama (2000) Ultrastructural observation of diapause and non-diapause eggs in the Band-Legged Ground Cricket, *Dianemobius nigrofasciatus. Zool. Sci.*, **17** (supplement), 68.

Umeya, Y. (1946) Embryonic hibernation and diapause in insects from the viewpoint of the hibernating eggs of the silkworm. *Bull. Seric. Exp. Sta. Jpn.*, **12**, 393–480. (in Japanese with English summary).

Umeya, Y. (1950) Studies on embryonic hibernation and diapause in insects. *Proc. Jpn. Acad.*, **26**, 1–9.

Williams, F. X. (1916) Photogenic organs and embryology of lampyrids. *J. Morphol.*, **28**, 145–207.