

Developmental Characteristics and Life History of the Korean Native Firefly, *Pyrocoelia rufa*

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Development and life history of the Korean native firefly, *Pyrocoelia rufa*, were investigated throughout the indoor rearing. Average size of egg with an oval shape was 1.7 mm and the hatchability of egg of *P. rufa* was approximately 88.5%. The larvae were pupated at the 5th instar and the body length of the matured larvae was 27.1 mm. The total periods of larval stage to the end of the 5th instar were approximately 104.7 days. Average pupal period was 10.3 days and average number of eggs oviposited by a female was 87.3 eggs. The body size of female in the pupa and adult was larger than that of male. The wings of female adult were deteriorated.

Key words: Insect, Firefly, *Pyrocoelia rufa*, Development, Life history

Introduction

Fireflies are well-known luminous beetles, which emit flashes with species-specific duration and frequency as signals for mating and hunting (Lloyd, 1983). Approximately 2,000 firefly species are found worldwide, except for the South Pole and the North Pole (Minami, 1983). Firefly luciferase catalyses the oxidative decarboxylation of D-luciferin in the presence of ATP and thereby light is emitted (Lembert, 1996). The firefly luciferase genes have been

studied deeply in some species (Cho *et al.*, 1999; Choi *et al.*, 2002, 2003; Devine *et al.*, 1993; De Wet *et al.*, 1987; Lee *et al.*, 2001; Masuda *et al.*, 1989; Ohiyama *et al.*, 1995; Tatsumi *et al.*, 1992) and are increasingly used as a highly-effective reporter gene in many organisms (DiLella *et al.*, 1988; Howard *et al.*, 1988; Jacobs *et al.*, 1993; Kondo *et al.*, 1992; Miller *et al.*, 1992; Vikas *et al.*, 1995).

The *Pyrocoelia*-group in the firefly is divided into two lineages. The first one consists of *P. rufa*, *P. miyako* and *P. atripennis*, and the second of *P. fumosa*, *P. oshimana*, *P. matsumurai matsumurai*, *P. m. kumejimensis*, *P. discicollis* and *P. abdominalis* (Suzuki, 1997, 2001). The body sizes of the former group are larger than those of the other Lampyrine species and members of the group are characterized by the continuous broadcast of strong light. *P. rufa* has the largest luminescent organs among the former group (Suzuki, 1997).

In Korea, three species have been reported as major fireflies: *Luciola lateralis* and *Hotaria unmunzana* belonging to Luciolinae, and *P. rufa* belonging to Lamyrinae (Choi *et al.*, 2002, 2003; Kim *et al.*, 2001; Lee *et al.*, 2001). Of these major fireflies, *P. rufa* is an abundant firefly species in Korea and is also found in China and only at Tsushima in the case of Japan. The luciferase gene of *P. rufa* has been reported (Lee *et al.*, 2001). Furthermore, population genetic structure of *P. rufa* was determined by mitochondrial cytochrome oxidase subunit I gene sequences (Lee *et al.*, 2003). However, ecological characteristics concerning the development and life history of the Korean native firefly, *P. rufa*, are not reported yet.

We have investigated some ecological information of the Korean native firefly, *P. rufa*. In this paper, the development characteristics and life history of *P. rufa* are described.

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Materials and Methods

Insects and indoor rearing

Adults of the Korean native firefly, *Pyrocoelia rufa*, were collected at Yangpeong and Miryang, Korea. The collected adult fireflies of *P. rufa* were reared in a plastic container (diameter, 8.7 cm; height, 2.0 cm) for mating and oviposition. Female and male adults with a ratio of 1:1 were placed in a plastic container. The plastic container for mating and oviposition was added with lichen and soil for the wet condition, and incubated at 25°C and 75 ± 5.0% R. H. After oviposition, the egg was incubated at 25°C for 2 months, at 7.5°C for 5 months, and then at 25°C under the 75 ± 5.0% R. H. and natural photoregime. The hatched larvae were reared in a device (20 × 40 × 10 cm) for larvae rearing. The device for larvae rearing was added with soils, lichen and pebbles for the favorable condition for rearing, and periodically sprayed with water for the wet condition. The diet for larvae of *P. rufa* was used with *Acusta despecta* as needed. The device for larvae rearing was incubated at 25°C under a natural photoregime. For the pupation and emergence, the larvae were continuously kept in the same device with population of approximately 20 larvae per device. The device for pupation and emergence was incubated at 25°C under the 75 ± 5.0% R. H. and natural photoregime.

Development and life history

The size of egg, larva, pupa and adults of *P. rufa* was measured with micrometer at a magnification. The average numbers of eggs laid and hatchability were examined. The

larval development of *P. rufa* was measured by larval size. The larval period of each instar was also measured. The morphology of the egg, larva, pupa and adult was photographed with a stereo microscope. The size of pupae and adults was sexually measured. Pupation and emergence rates were examined. The period and longevity of pupae and adults were respectively measured.

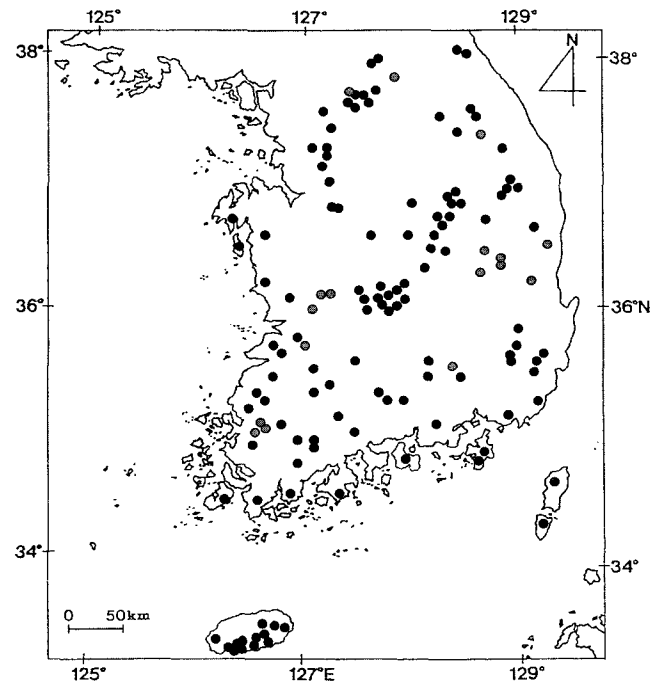


Fig. 1. Map of Korean peninsula showing distributions of the firefly, *P. rufa*.



Fig. 2. Examples of micro-habitats of the firefly, *P. rufa*.

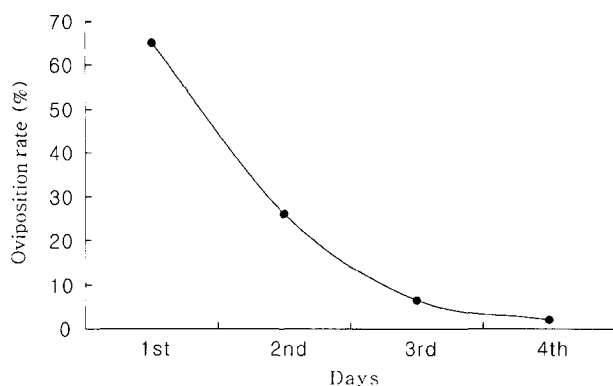


Fig. 3. Daily oviposition rate of the female adult of *P. rufa*.

Results and Discussion

The Korean native firefly, *P. rufa*, is widely distributed in Korea and only at Tsushima in the case of Japan (Fig. 1). *P. rufa* is known to occur in various habitats such as stream area, wet fields and mountain area (Fig. 2).

After mating, a female adult laid eggs during 4 days and most of its oviposition occurred at the 1st day (65.2%) and 2nd day (26.1%) (Fig. 3). Average fecundity from a female adult mated was approximately 87.3 ± 26.3 eggs (Table 1). The shape of egg of *P. rufa* was an oval and the color of the just-oviposited egg was yellow white, changing into thick brown with embryogenesis. The egg size of *P. rufa* was estimated as 1.7 ± 0.1 mm (Table 1). The hatchability of *P. rufa* eggs was approximately 88.5% (Table 1).

The growth of larvae in terms of larval body length was significantly increased with a larval development (Table 2). The larval length from 1st instar to 5th instar was ranged from 7.6 mm to 27.1 mm. The span of each instar was also measured (Table 3). Particularly, the span of 5th instar was prolonged over one month. Consequently, the total periods of larval stage to the end of the 5th instar covered approx-

Table 1. Number of egg laid, hatchability and egg size of *P. rufa*

Number of egg laid per a female	Hatchability (%)	Egg size (mm)
87.3 ± 26.3	88.5 ± 14.9	1.7 ± 0.1

Table 2. Body length of each instar of *P. rufa* larva

Body length of each instar (mm)				
1st	2nd	3rd	4th	5th
7.6 ± 0.4	10.5 ± 0.7	15.8 ± 1.1	20.9 ± 1.4	27.1 ± 3.2

Table 3. Larval period of each instar of *P. rufa*

Duration of each instar (Days)					
1st	2nd	3rd	4th	5th	Total
11.5 ± 7.3	19.1 ± 7.7	14.5 ± 6.6	16.2 ± 4.7	41.3 ± 11.4	104.7 ± 17.6

Table 4. Pupation rate, emergence rate and mortality of *P. rufa* in indoor rearing

Pupation rate (%)	Pupal period (Days)	Emergence rate (%)	Mortality (%)		Rate of non-pupated larvae (%)
			Larvae	Pupae	
62.5	10.3 ± 1.0	55.8	19.6	6.7	17.9

Table 5. Body length of the pupa and adult of *P. rufa*

Pupa (mm)		Adult (mm)	
Female	Male	Female	Male
19.2 ± 2.0	14.9 ± 1.5	19.4 ± 3.1	15.3 ± 1.5

imately 104.7 days. The larval size of last instar of *P. rufa* in this study is approximately 27.1 mm, as reported in that the body size of *P. rufa* is larger than that of the other Lampyrine species (Suzuki, 1997). One of the major fireflies in Korea, the body size of last instar of *L. lateralis* was reported as approximately 15.8 mm (Kim *et al.*, 2001).

The mature larvae were mostly pupated under the lichen or pebbles. The pupation rate was approximately 62.5% and the pupal period was approximately 10.3 days (Table 4). The mortality in the larval or pupal stage and the non-pupated larvae were correlated with pupation and emergence rate (Table 4). The non-pupated larvae were approximately 17.9%. *P. rufa* in the indoor rearing shows the mortalities with 19.6% during the larval stage and with 6.7% during the pupal stage. Regarding sexual difference in the body size of pupae, the body length of female was larger than that of male. According to the measurements, the average length of female and male pupae was 19.2 mm and 14.9 mm, respectively (Table 5). The external morphology of the pupae was shown in Fig. 4C.

The body length of female and male adults was 19.4 mm and 15.3 mm, respectively (Table 5). The body size of female adult was larger than that of male. Regarding sexual difference in the external morphology of adults, the wings of female were deteriorated (Fig. 4D).

Life history of *P. rufa* is composed of distinct four developmental stages of egg, larva, pupa and adult (Fig. 4 and 5). Life cycle during two successive generations of *P. rufa* at 25°C under a natural photoperiod was described in Fig. 5. Egg period was September in this year to May in the next year. The total periods of egg stage covered usually about 9 months, indicating that most of its life cycle consists of egg stage. Larval period was from June to August, but larvae with a generation in two years were observed from September in this year to May in the next year. Pupal period was July to September and adult period was August to September.

This study elucidated the developmental characteristics

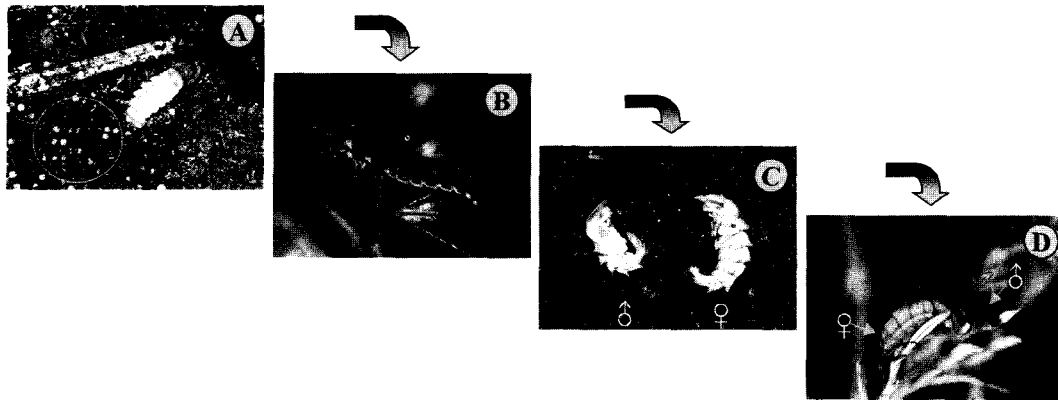


Fig. 4. Life cycle of the firefly, *P. rufa*. A, egg; B, larva; C, pupa; D, adult.

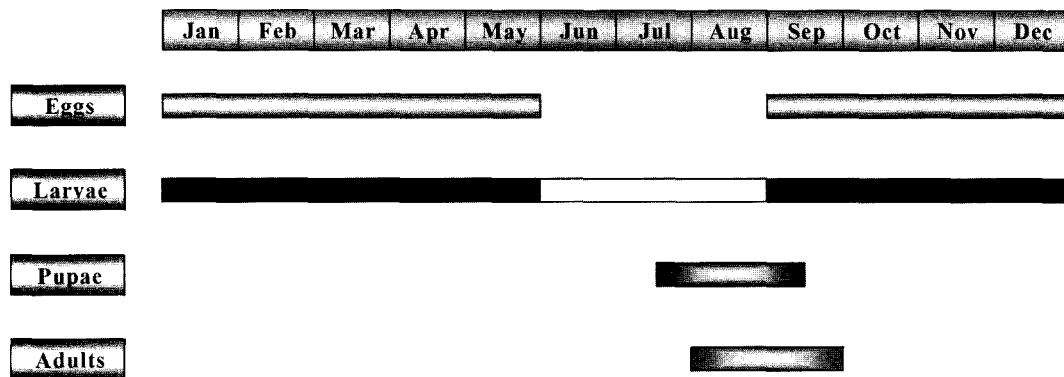


Fig. 5. Mode of monthly occurrence of the firefly, *P. rufa*, with different developmental stages. Open bar in the larval stage indicates the larval period of the firefly with two-year cycle.

and life history of *P. rufa* for two generations. These results have shown that the indoor rearing of *P. rufa* is possible. An increased understanding of development and life history of *P. rufa* should provide further information for the ecological and physiological study.

References

- Cho, K. H., J. S. Lee, Y. D. Choi and K. S. Boo (1999) Structural polymorphism of the luciferase gene in the firefly, *Luciola lateralis*. *Insect Mol. Biol.* **8**, 193-200.
- Choi, Y. S., K. S. Lee, J. S. Bae, K. M. Lee, S. R. Kim, I. Kim, S. M. Lee, H. D. Sohn and B. R. Jin (2002) Molecular cloning and expression of a cDNA encoding the luciferase from the firefly, *Hotaria unmunzana*. *Comp. Biochem. Physiol.* **132B**, 661-670.
- Choi, Y. S., J. S. Bae, K. S. Lee, S. R. Kim, I. Kim, J. G. Kim, K. Y. Kim, S. E. Kim, H. Suzuki, S. M. Lee, H. D. Sohn and B. R. Jin (2003) Genomic structure of the luciferase gene and phylogenetic analysis in the *Hotaria*-group fireflies. *Comp. Biochem. Physiol.* **134B**, 199-214.
- Devine, J. H., G. D. Kutuzova, V. A. Green, N. N. Ugarova and T. O. Baldwin (1993) Luciferase from the east European firefly *Luciola mingrelica*: cloning and nucleotide sequence of the cDNA, overexpression in *Escherichia coli* and purification of the enzyme. *Biochim. Biophys. Acta* **1173**, 121-132.
- De Wet, J. R., K. W. Wood, M. DeLuca, D. R. Helinski and S. Subramani (1987) Firefly luciferase gene: structure and expression in mammalian cells. *Mol. Cell Biol.* **7**, 725-737.
- DiLella, A. G., D. A. Hope, H. Chen, M. Trumbauer, R. J. Schwartz and R. G. Smith (1988) Utility of firefly luciferase as a reporter gene for promoter activity in transgenic mice. *Nucleic Acids Res.* **16**, 4159.
- Howard, P. K., K. G. Ahern and R. A. Firtel (1988) Establishment of a transient expression system for *Dictyostelium discoideum*. *Nucleic Acids Res.* **16**, 2613-2623.
- Jacobs, W. R., R. G. Barletta, R. Udami, J. Chan, G. Kalkut, G. Sosne, T. Kieser, G. J. Sarkis, G. F. Hatfull and B. R. Bloom (1993) Rapid assessment of drug susceptibilities of *Mycobacterium tuberculosis* by means of luciferase reporter phages. *Science* **260**, 819-822.
- Kim, J. G., S. E. Kim, J. Y. Choi, H. J. Yoon, Y. C. Choi, N. Ohba, B. R. Jin and S. K. Noh (2001) Developmental characteristics and life history of the Korean native firefly, *Luciola lateralis*. *Int. J. Indust. Entomol.* **3**, 141-147.
- Kondo, T., N. Takahashi and M. Muramatsu (1992) The regu-

- lation of the murine Hox-2.5 gene expression during cell differentiation. *Nucleic Acids Res.* **20**, 5729-5735.
- Lee, K. S., H. J. Park, J. S. Bae, T. W. Goo, I. Kim, H. D. Sohn and B. R. Jin (2001) Molecular cloning and expression of a cDNA encoding the luciferase from the firefly, *Pyrocoelia rufa*. *J. Biotechnol.* **92**, 9-19.
- Lee, S. C., J. S. Bae, I. Kim, H. Suzuki, S. R. Kim, J. G. Kim, K. Y. Kim, W. J. Yang, S. M. Lee, H. D. Sohn and B. R. Jin (2003) Mitochondrial DNA sequence-based population genetic structure of the firefly, *Pyrocoelia rufa* (Coleoptera: Lampyridae). *Biochemical Genetics* In press.
- Lembert, N. (1996) Firefly luciferase can use L-luciferin to produce light. *Biochem. J.* **317**, 273-277.
- Lloyd, J. E. (1983) Bioluminescence and communication in insects. *Annu. Rev. Entomol.* **28**, 131-160.
- Masuda, T., H. Tatsumi and E. Nakano (1989) Cloning and sequence analysis of cDNA for luciferase of a Japanese firefly, *Luciola cruciata*. *Gene* **77**, 265-270.
- Miller, A. J., S. R. Short, N. H. Chua and S. A. Say (1992) A novel circadian phenotype based on firefly luciferase expression in transgenic plants. *Plant Cell* **4**, 1075-1087.
- Minami, K. (1983) Studies on the firefly. pp. 7-12, Scientist Co., Tokyo.
- Ohmiya, Y., N. Ohba, H. Toh and F. I. Tsuji (1995) Cloning, expression and sequence analysis of cDNA for the luciferases from the Japanese fireflies, *Pyrocoelia miyako* and *Hotaria parvula*. *Photochem. Photobiol.* **62**, 309-313. .
- Suzuki, H. (1997) Molecular phylogenetic studies of Japanese fireflies and their mating systems (Coleoptera: Cantharodea). *Tokyo Metro. Univ. Bull. Nat. Hist.* **3**, 1-53.
- Suzuki, H. (2001) Studies on biological diversity of firefly in Japan. *Int. J. Indust. Entomol.* **2**, 91-105.
- Tatsumi, H., N. Kajiyama and E. Nakano (1992) Molecular cloning and expression in *Escherichia coli* of a cDNA clone encoding luciferase of a firefly, *Luciola lateralis*. *Biochim. Biophys. Acta* **1131**, 161-165.
- Vikas, B. P., S. Sumathy and P. G. Karumathil (1995) Baculovirus mediated high-level expression of luciferase in silkworm cells and larvae. *BioTech.* **19**, 97-104.