

Studies on Biological Diversity of Firefly in Japan

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Taxonomic and phylogenetic studies of firefly in Japan have been reviewed. Forty-six lampyrid species and one rhagophthalmid are distributed in the Japanese Islands including the Ryukyus. Recently, molecular phylogenetic approaches have been employed in the systematic study of firefly using mitochondrial and luciferase genes. Based on the molecular phylogenetic trees, evolutionary process of flashing patterns related strictly to mating behavior was estimated. Furthermore, genetic diversity studies revealed geographic differentiation patterns within species, and conservation measures of firefly were proposed to protect genetic resources endemic to the localities.

Key words : Lampyridae, Rhagophthalmidae, Taxonomy, Phylogeny, Phylogeography, Conservation measures, Japan

Introduction

The firefly has been well known as a luminous insect since old days, and people have been fascinated by a multiple display of the lights. In the Occidental world, bioluminescent lights has been studied scientifically not only in firefly but also in other luminous organisms such as bacteria, fungi, jellyfish, snail, squid, fish and others (reviewed by Harvey, 1952). In the Oriental world, on the other hand, people have believed that luminous insects were the incarnation of the soul, and insect bioluminescence affected human life mentally. In China, Korea, and Japan, firefly was personified and composed in literatures frequently (reviewed by Kanda, 1935, 1981; Hazama, 1943; Konishi,

1977, 1992, 1997). In 1900, Watasé presented the orientation of firefly studies scientifically at first in Japan and illustrated American species, *Photinus consanguineus*, *Photuris pennsylvanica*, and *Pyrocoelia lucifera*, collected in Woods Hole by himself. He (1902) also introduced fireflies from the world and Japan, and interpreted bioluminescent phenomena biologically. *Pyrocoelia rufa* distributed in Japan and adjacent regions was reported by Watasé (1904). Okada (1928) presented detail morphology of aquatic larvae of the Japanese species, *Luciola cruciata* and *L. lateralis*, to the world. Kanda (1935, 1981) made a great advance in firefly biology, e.g. morphology, ecology, life cycle, and physiological chemistry of light production, and listed classical literatures composing firefly. Since then, many books about firefly have been published in Japan (e.g. Hara, 1940; Hazama, 1943; Minami, 1961, 1983; Yajima and Ogino, 1980; Nakane and Ohba, 1981; Kuribayashi, 1979; Takeuchi, 1985; Ohba, 1986, 1988; Haneda, 1990; Mitsuishi, 1990, 1996; Fukaishi, 1997). Drs. Haneda and Ohba in particular contribute many papers of luminous organisms including firefly to the journal, Science Report of the Yokosuka City Museum, issuing from 1956.

In this paper, studies on biological diversity of firefly in Japan are reviewed, especially taxonomy, phylogeny relation to mating behavior, and conservation measures.

Taxonomy

Taxonomic studies of fireflies were made by such entomologists as Laporte (1833), Motschulsky (1845, 1852, 1853, 1854), LeConte (1850, 1852, 1881), Gorham (1880, 1883), Olivier (1885), and others in the 19th century. Olivier (1907, 1910a) established a classification of the family Lampyridae in his catalogs as Fascicule 53 of Wytzmanis Genera Insectorum and in Pars 9 of the Schenkling-Junk Coleopterorum Catalogus. He enumerated 1,109 species

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in 58 genera belonging to nine subfamilies, Lamprocerinae, Lucidotinae, Dadophorinae, Photininae, Lampyrinae, Megalophthalminae, Amydetinae, Luciolinae and Photurinae (Olivier, 1910a). Pic (1911, 1955) described many species from the world in the 1910s to 1950s. Wittmer (1939, 1944, 1948, 1979) reviewed the superfamily Cantharoidea including Lampyridae. After that, some genera of Lycidae and Drilidae were moved to Lampyridae. Green (1948) published his outline for a revision of the Nearctic Lampyridae, and in 1956, 1957, 1959, and 1961 he revised the North American species of *Photinus*, *Pyractomena*, *Microphotus*, and *Pyropyga*, respectively. In 1964, McDermott made a great advance in taxonomy of the Lampyridae showing his outline for a revision of the world species, and two years later he published a catalog of the Lampyridae as Pars 9 of the Junk's Coleopterorum Catalogus, which is the second and revised edition of Olivier's Catalogus (McDermott, 1966). In this catalog, he recognized seven subfamilies, Lampyrinae, Photurinae, Pterotinae, Luciolinae, Amydetinae, Matheteinae, and Rhagophthalminae, and listed 1891 existing world species and four fossil ones in 86 genera and 8 subgenera. In the 1960's to 1980's, Ballantyne (1968, 1987a, 1987b, 1988), Ballantyne and Mclean (1970), and Ballantyne and Buck (1979) revised the Australian, Indomalayan, and New Guinean Luciolini. Crowson (1972) revised the classification of the superfamily Cantharoidea, and considered that McDermott's subdivisions within the Lampyridae were not natural. At that time he proposed a revised system including new categories based on adult males: The Matheteinae and Rhagophthalminae in McDermott's system were transferred from those of the Lampyridae to different families, the Omethidae and Phengodidae, respectively, and the subfamilies Otoretadrilinae, Cyphonocerinae, and Otoretinae in the Lampyridae were newly established. Geisthardt (1983a, 1983b, 1984, 1987) revised the European Lampyrinae. In a decade, Zaragoza Caballero (1993, 1995) described a lot of new species from Mexico. Jeng *et al.* (1998a, 1998b, 1999a, 1999b) reviewed and described Taiwanese species of *Cyphonocerus*, *Curtos* and *Pyrocoelia*. Further they noticed that *Psilocladinae* is the valid name of the subfamily, and *Cyphonocerinae* should be a subjective synonym of the former (Jeng *et al.*, 1998a). Lai *et al.* (1998) presented a check-list of Taiwanese firefly and enumerated 44 species of 11 genera in the four subfamilies, Otoretinae, Luciolinae, Lampyrinae, and *Psilocladinae*. Kawashima (2000) redescribed *Rhagophthalmus ingens* from Northern Vietnam and established new genus *Menghuoius* in the family Rhagophthalmidae.

The Japanese species of firefly were studied by foreign researchers, Thunberg (1784), Motschulsky (1854, 1860,

1866), Kiesenwetter (1874, 1879), Gorham (1880, 1883), Olivier (1886) and Lewis (1895, 1896) in the early stage. Matsumura (1918, 1928) listed 22 and 25 firefly species including 10 and 4 new ones, respectively in the Japanese Islands, Taiwan, Korean Peninsula, and Sakhalin. Okada (1928) pointed out that *Luciola picticollis* Kiesenwetter 1874 and *L. vitticollis* Kiesenwetter 1874 were synonym of *L. cruciata* Motschulsky 1854 and *L. lateralis* Motschulsky 1860, respectively, and he also presented a check-list of fireflies in the Japanese Islands and adjacent regions (33 species), in which he enumerated only ten species from Japan without Matsumura's new ones, *viz.* *Psilocladus variolosus*, *Lucidina biplagiata*, *L. accensa*, *Pyrocoelia atripennis*, *P. rufa*, *P. discicollis*, *P. fumosa*, *Luciola cruciata*, *L. lateralis* and *L. parvula* (Okada, 1931). Yuasa (1937) proposed a new genus *Hotaria* which is closely related to *Luciola*, and *Luciola parvula* was combined with *Hotaria*. Nakane (1947) pointed out that *Psilocladus variolosus* is identical with *Cyphonocerus ruficollis* and treated the genus *Cyphonocerus* as a synonym of *Psilocladus*. In 1949, Nakane and Ohbayashi described a new member of *Lucidina*.

In the 1960's to 1980's, Nakane (1968a), Satô (1978), and Nakane and Ohba (1981) enumerated 24, 34, and 39 species, respectively as the Japanese members on account of discoveries of new species mainly from the Ryukyu Islands (Nakane, 1961, 1963, 1967, 1970, 1968b-1979, 1981, 1983, 1985, 1987; Ohbayashi and Satô, 1963; Satô, 1968, 1976, 1986; Chûjô and Satô, 1972). Although Nakane (1947) considered the genus *Cyphonocerus* to be a synonym of *Psilocladus*, later in 1967 he regarded the former as a valid genus (Nakane, 1967). McDermott (1966) put the genus *Hotaria* into subgenus of *Luciola* in his catalog. Ballantyne (1968) also could not resolve the position of *Hotaria* in her revisional studies of Luciolini and revised it the subgenus of *Luciola*. Chûjô and Satô (1970a) moved *Luciola costipennis* to *Curtos*, and they reviewed the Japanese and Taiwanese species of *Curtos* and presented a check-list of *Curtos* species known from the world (Chûjô and Satô, 1970b).

In a decade, some articles have been devoted to the taxonomy of the Japanese fireflies, and several new species and subspecies have been added to the Japanese fauna (Satô, 1991; Satô and Kimura, 1994; Wittmer and Ohba, 1994; Jeng *et al.*, 1998a, 1998b). It is remarkable that the rhagophthalmid species, *Rhagophthalmus ohbai*, was discovered from the Ryukyu Islands (Wittmer and Ohba, 1994). The distribution range of the genus is mainly the Continental Oriental region. Specific name of *Curtos okinawana* Matsumura was altered as *C. okinawanus* by mandatory change (Jeng *et al.*, 1998b). Recently, Ohba (1998) listed 46 lampyrid species and one rhagophthalmid

from Japan. However, *Luciola japonica* Thunberg and *L. praeusta* Kiesenwetter were included in this list. According to Matsumura (1918), Okada (1931), and Ohba (1986), the occurrence of these two species in Japan is very doubtful. After then, two new species of *Stenocladus* were described from the Ryukyu and Amami Islands (Kawashima, 1999). If we consider the doubtful species to be excluded from the list, it seems reasonable to suppose that at present, 46 lampyrid species and one rhagophthalmid are distributed in the Japanese Islands including the Ryukyus.

Classification

In regard to the taxonomy of the Lampyridae, Crowson's (1972) treatment of the subfamilies was adopted, with slight modifications (Jeng *et al.*, 1998a). However, the genus *Rhagophthalmus* is very problematical. Olivier (1910 b) included the three genera, *Dioptoma*, *Ochotyra*, and *Rhagophthalmus* in the family Rhagophthalmidae. One year later, he treated *Rhagophthalmus* as a member of the Lampyridae (Olivier, 1911). Crowson (1955, 1967) gave the subfamily Rhagophthalminae in the Lampyridae, and this treatment was followed by McDermott (1964, 1966). In 1972, Crowson treated *Rhagophthalmus* as a member of the Phengodidae, and Wittmer in Wittmer and Ohba (1994) placed it in the Rhagophthalmidae. Thus, the taxonomic position of *Rhagophthalmus* is not yet clear. In this paper, for the time being, Wittmer's taxonomic treatment of this genus is adopted, because it is the most recent. Accordingly, classification of the Japanese fireflies is listed in Table 1.

Phylogeny

A considerable number of taxonomic studies have been made on fireflies (Olivier, 1907, 1910a; McDermott, 1964, 1966 and others), and nowadays, about 2,000 species are known from the world. Nevertheless, little attention has been given to the phylogenetic relationships of fireflies. In respect of this point, Crowson (1972) referred to the taxonomic relationships between subfamilies and the context of the phylogeny of the Cantharoidea. Wilkerson and Lloyd (1975) applied paper chromatography of fluorescent compounds to the systematics of the North American fireflies, and revealed generic and species differences of 2-dimensional chromatographic pattern of the fluorescent pigment. However, phylogenetic relationships among them were not presented.

Recently, molecular phylogenetic approaches have been

Table 1. A list of fireflies found in Japan

Superfamily Cantharoidea
Family Lampyridae
Subfamily Lampyrinae
Genus <i>Lucidina</i> Gorham, 1883
<i>L. accensa</i> Gorham, 1883
<i>L. biplagiata</i> (Motschulsky, 1866)
<i>L. natsumiae</i> Chûjô et M. Satô, 1972
<i>L. okadai</i> Nakane et Ohbayashi, 1949
Genus <i>Pyrocoelia</i> Gorham, 1880
<i>P. abdominalis</i> Nakane, 1979
<i>P. atripennis</i> Lewis, 1896
<i>P. discicollis</i> (Kiesenwetter, 1874)
<i>P. fumosa</i> (Gorham, 1883)
<i>P. iriomotensis</i> Nakane, 1985
<i>P. matsumurai</i> Nakane, 1963
<i>P. m. kumejimensis</i> Chûjô et M. Satô, 1972
<i>P. miyako</i> Nakane, 1981
<i>P. oshimana</i> Nakane, 1985
<i>P. rufa</i> E. Olivier, 1886
Genus <i>Pristolytus</i> Gorham, 1883
<i>P. sagulatus</i> Gorham, 1883
<i>P. s. adachii</i> M. Satô, 1986
<i>P. s. amami</i> Nakane, 1961
<i>P. shikokensis</i> Ohbayashi et M. Satô, 1963
Subfamily Luciolinae
Genus <i>Curtos</i> Motschulsky, 1845
<i>C. costipennis</i> (Gorham, 1880)
<i>C. okinawanus</i> Matsumura, 1918
Genus <i>Luciola</i> Laporte, 1833
<i>L. cruciata</i> Motschulsky, 1854
<i>L. c. towadensis</i> Nakane, 1987
<i>L. kuroiwae</i> Matsumura, 1918
<i>L. lateralis</i> Motschulsky, 1860
<i>L. owadai</i> M. Satô et Kimura, 1994
<i>L. yayeyamana</i> Matsumura, 1918
<i>L. (Hotaria) parvula</i> Kiesenwetter, 1874
<i>L. (Hotaria) tsushimana</i> Nakane, 1970
Subfamily Otoretinae
Genus <i>Drilaster</i> Kiesenwetter, 1879
<i>D. anomalus</i> Nakane, 1979
<i>D. akusekianus</i> Nakane, 1983
<i>D. axillaris</i> Kiesenwetter, 1879
<i>D. bicolor</i> M. Satô, 1968
<i>D. flavicollis</i> Nakane, 1979
<i>D. fuscicollis</i> Nakane, 1979
<i>D. iokii</i> M. Satô, 1968

Table 1. Continued

<i>D. ohbayashii</i> M. Satô, 1968
<i>D. okinawensis</i> Nakane, 1979
<i>D. shibatai</i> M. Satô, 1968
<i>D. unicolor</i> Lewis, 1895
Genus <i>Stenocladus</i> Fairmaire, 1878
<i>S. azumai</i> Nakane, 1981
<i>S. flavipennis</i> Kawashima, 1999
<i>S. shirakii</i> Nakane, 1981
<i>S. yoshikawai</i> Nakane, 1981
<i>S. yoshimasai</i> Kawashima, 1999
Subfamily Psilocladinae
Genus <i>Cyphonocerus</i> Kiesenwetter, 1879
<i>C. inelegans</i> Nakane, 1967
<i>C. marginatus</i> Lewis, 1895
<i>C. okinawanus</i> Nakane, 1983
<i>C. o. amamianus</i> Jeng, Yang et M Satô, 1998
<i>C. ruficollis</i> Kiesenwetter, 1879
<i>C. watarii</i> M. Satô, 1991
<i>C. yayeyamensis</i> M. Satô, 1976
Family Rhagophthalmidae
Genus <i>Rhagophthalmus</i> Motschulsky, 1853
<i>R. ohbai</i> Wittmer, 1994

employed in the systematic study of firefly. Suzuki *et al.* (1996a) presented phylogenetic relationships of Japanese species of the subfamily Luciolinae employing allozyme analysis and discussed the interrelation between speciation and flash communication systems. However, the Nei's (1972) genetic distances (D) between genera were greater than 2.0. Since large D value ($D > 1$) contains considerable error, it is impossible to estimate the branching order exactly of the phylogenetic tree, even though many genetic loci are studied (Nei, 1987). Therefore, the phylogenetic tree of the lucioline species deduced from allozyme analysis was not satisfactory.

One year later, Suzuki (1997a, 1997b) presented phylogenetic trees based on mitochondrial (mt) 16S ribosomal RNA gene of 27 taxa of the Japanese Lampyridae belonging to four subfamilies and one species of Rhagophthalmidae (Fig. 1) and discussed evolutionary divergence of their mating systems. *Calochromus rubrovestitus* of the family Lycidae was used as an outgroup species. The phylogenetic tree shows major five lineages, the lucioline, *Cyphonocerus*, lampyrine, *Stenocladus*-*Rhagophthalmus*, and *Drilaster* groups. These grouping are almost consistent with taxonomic treatment of the subfamilies. In the subfamily Luciolinae, five lineages are recognized. The first lineage consists of *Luciola* (*Hotaria*) *parvula*

and *L. (H.) tsushimana*. These are closely related to *L. unmunsana* distributed widely in the Korean Peninsula (Suzuki *et al.*, in preparation). The second of *L. cruciata*, *L. owadai*, and *L. lateralis*, which are aquatic in larval stage. The third of *Curtos costipennis* and *C. okinawanus*. The fourth of only *L. yayeyamana* and fifth of only *L. kuroiwae*. *L. filiformis* distributed in Taiwan and *Pteroptyx* species widely in Southeast Asia are clustered with the fourth and fifth lineages, respectively (Suzuki *et al.*, in preparation).

The subfamily Lampyrinae is divided into three lineage, the *Pyrocoelia*, *Lucidina* and *Pristolycus* lineages. The *Pristolycus* is genetically differentiated from *Pyrocoelia* and *Lucidina*, but included in the lucioline cluster. Morphologically *Pristolycus* is heterogeneous in the Lampyrinae that the head of *Pristolycus* is partially exposed in front of the pronotum, but others in which it is normally completely hidden. However, *Pristolycus* is obviously different from the lucioline species in morphology and ecology. Therefore, it is necessary to reconfirm the genetic similarity between *Pristolycus* and Luciolinae by other mitochondrial and nuclear genes. The *Pyrocoelia* lineage is divided into two sublineages. One sublineage consists of *P. rufa*, *P. miyako*, and *P. atripennis*, and the other of *P. fumosa*, *p. oshimana*, *P. matsumurai matsumurai*, *P. m. kumejimensis*, *P. discicollis*, and *P. abdominalis*. The *Lucidina* lineage consists of *L. biplagiata*, *L. accensa* and *L. okadai*.

In the subfamily Psilocladinae, only *Cyphonocerus ruficollis* was analyzed. The *Cyphonocerus* is rooted with the Lampyrinae, but the bootstrap value is not significantly high (58%). In the subfamily Otoretinae, *Drilaster* and *Stenocladus* are not clustered, but *Stenocladus* and *Rhagophthalmus ohbai* of the Rhagophthalmidae form a cluster. On the other hand, *Drilaster* is rooted at the base of the lampyrid group. Therefore, *Rhagophthalmus* is not differentiated significantly to distinguish different family from Lampyridae in the mt 16S r-DNA sequences. However, interrelationships among the five lineages are not clarified significantly because of low bootstrap values.

Luciferase gene of some beetles has been cloned and characterized in the course of the study of spectral diversity of bioluminescence (e.g. De Wet *et al.* 1985, 1987; Wood *et al.* 1989; Masuda *et al.* 1989; Tatsumi *et al.* 1992; Devine *et al.* 1993; Ohmiya *et al.* 1995; Sala-Newby *et al.* 1996; Li *et al.* 1997; Cho *et al.* 1999). Ohmiya *et al.* (2000) compared amino acid sequences of luciferase among 13 species distributed in four families, Elateridae, Lampyridae, Phengodidae, and Rhagophthalmidae, and pointed out that *Rhagophthalmus ohbai* is more closely related to the members of Phengodidae than those of Lampyridae.

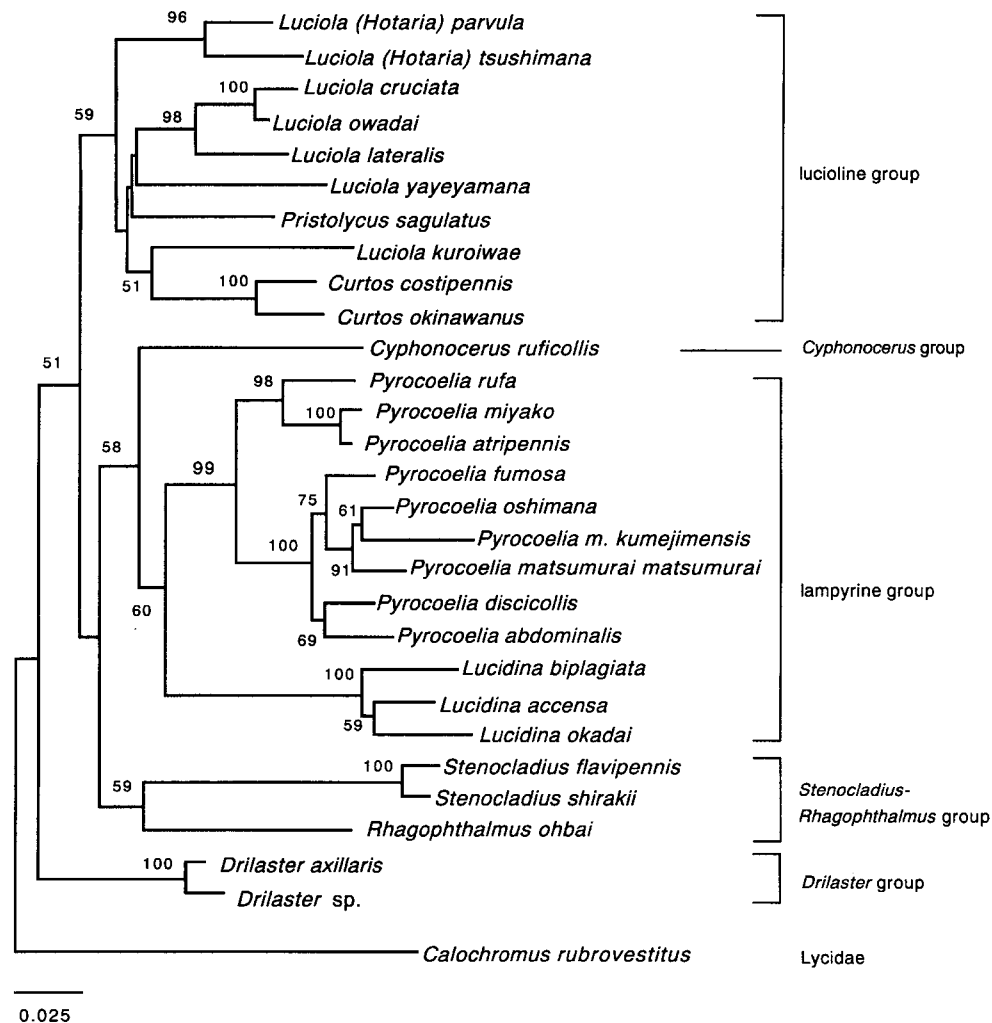


Fig. 1. A phylogenetic tree of Japanese fireflies constructed from mitochondrial 16S ribosomal RNA gene by NJ method. Scale bar indicates 2.5% nucleotide divergence of Kimura's two-parameter model (Suzuki, 1997).

Mating system

The luminescent signal of the firefly has been recognized as an important method of sexual communication. Osten-Sacken (1861) and Emery (1886) referred to the sexual nature of firefly flashing experimentally working on *Photinus pyralis* and *Luciola italica*, respectively. After that, McDermott (1910, 1911, 1912, 1917), Mast (1912), and Barber (1951) reported light-emission of some American fireflies, and the function of the signals was interpreted as a mating adaptation. In ethological terms, it is called the flash communication system, and has been studied intensively by Lloyd (1964, 1965a, 1965b, 1966a, 1966b, 1972a, 1973a), Kaufman (1965), Papi (1969) and others. According to Lloyd (1971, 1978, 1983), the system is classified into two categories, System I and II, after Alexander's (1968) system of acoustical and visual signal of arthropods. The mating protocol of Lloyd's signal sys-

tem I is represented by the European firefly, *Lampyrus noctiluca*. In this species, a female broadcasts a continuous signal and a flying male recognizes it, and then he approaches her (glow system). Lloyd's signal system II, on the other hand, is represented by the North American firefly, *Photinus* species. In these *Photinus* species, a flying male emits a pulsating signal, a female recognizes it and responds to him with a signal. Then, the male recognizes her signal and approaches her (flash-answer system). In the latter system, inter-flash interval of the male and timing of the female's flash response are species-specific. In addition, Lloyd (1966a) considered the developing step of the systems from I to II. He (1972a) also reported a compound system represented by the New Guinean *Luciola* firefly as an additional system. In this system, mating protocol includes five distinct phases or stages: (1) sedentary signaling, (2) chasing, (3) walking-luminescing, (4) mounting, and (5) coupling. Synchron-

nous flashing behavior has been observed in the Oriental fireflies of the genus *Pteroptyx* (Reinking, 1921; Buck, 1935, 1938; Buck and Buck, 1966; Haneda, 1966; Lloyd, 1973a; Case, 1980). Lloyd (1973b) proposed a model for mating protocol of synchronous flashing, and physiological interpretations of the synchronous flashing were presented (Buck and Buck, 1968, 1978; Buck *et al.*, 1981; Buck, 1988). In addition to those, pheromonal communication of diurnal fireflies has also reported (Williams, 1917; Hess, 1920; Lloyd, 1972b).

In Japan, Yajima (1978) studied diurnal activities and luminous signals of *Luciola cruciata*, and showed an exchange of lights between male and female in mating. In 1983, Ohba classified mating systems into six categories, the HP, LC, LL, PR, CR, and LB systems, working with the Japanese firefly fauna. According to him, they are as follows. The HP system is represented by *Luciola (Hotaria) parvula* and corresponds to the Lloyd's signal system II. The LC system is represented by *Luciola cruciata*. In this species, flying males associate and emit flashes in synchrony or semisynchrony, and females recognize males' signals, though they are much smaller in number than the males, and females emit answering single-pulsed flashes; the males recognize the answering flashes and then approach the female; occasionally, the female responds to the flashes of the male in variable flash patterns while he is walking near her; thereafter they copulate. This system is similar to the compound signal system of Lloyd (1972a). In the LL system represented by *Luciola lateralis*, a flying male emits single-pulsed flashes; a female recognizes the flashes and begins to respond with distinctive flashes; the male approaches her and converts his flash pattern into twinkling flashes and directs the flashes to her. Both sexes continue emitting their distinctive flashes, and thereafter they copulate. Consequently, it may be said that a male and a female emit distinctive flashes and recognized each other's flash pattern. In this system, critical timing of female's flash response is not evident, but the flicker of light is a key signal to induce mating behavior. Moreover, males are attracted by artificial flash lights such as hazard blinkers of a car. In the PR system represented by *Pyrocoelia rufa*, a male is attracted by continuous light signals of a female, and copulating behavior is released by her pheromone. This system corresponds to Lloyd's signal system I. The CR system is represented by *Cyphonocerus ruficollis*, a diurnal species, but it shows copulating behavior in the twilight. In this species, male mating behavior is released by her pheromone. Weak luminescent light may function as a supplementary signal in this mating system, although its role is uncertain. The LB system, the last system of the Ohba's scheme, is represented by *Lucidina biplagiata*.

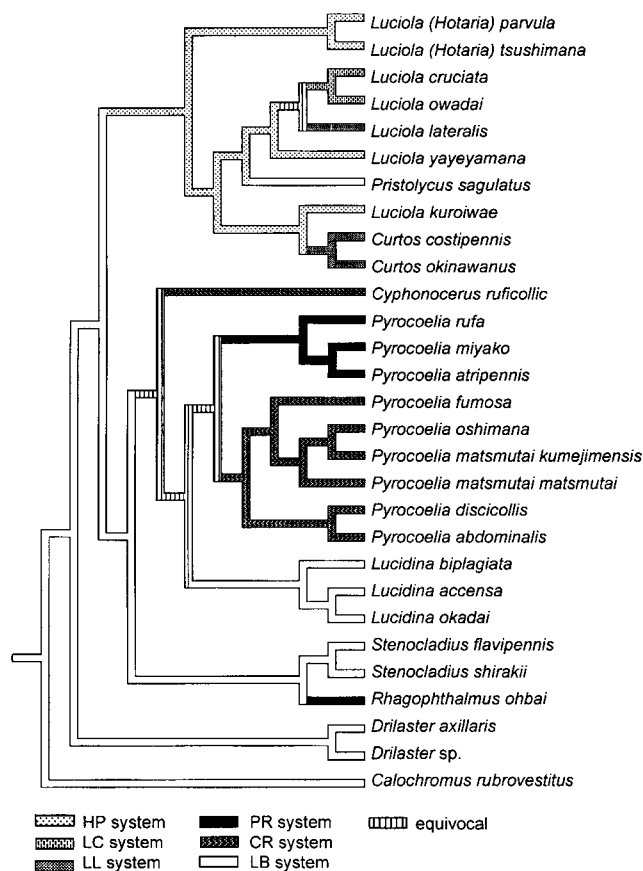


Fig. 2. Evolutionary tree of the Ohba's mating systems (HP, LC, LL, PR, CR, and LB systems) of Japanese fireflies (Suzuki, 1997).

This system is similar to the CR system, but no luminescent light is necessary for mating. The sex-attractant pheromone of *Pyrocoelia oshimana* was analyzed by gas chromatography mass spectrometry (Shibue *et al.*, 2000).

In Figure 2, Ohba's six mating systems are most parsimoniously allocated to each lineage of the mt-16S phylogenetic tree. The mating system of *Drilaster* which is rooted at the base of the lampyrid group is pheromonal (LB). Therefore, the pheromonal mating system is considered a primitive system, even though all the lampyrids are actively luminous in larval stage. In the lampyrine-*Cyphonocerus* lineage, continuous luminescence has been incorporated retaining a pheromonal mating system in adult. However, the tree does not show a divergent sequence between the CR and PR systems. On the other hand, it is considered that flash-answer system is derived from pheromonal system with some steps inside the luciline lineage. In this lineage, the pheromonal system has been lost and instead flash emission has been incorporated. After then, the species-specific flash patterns for mating (HP, LL, and LC systems) have been established.

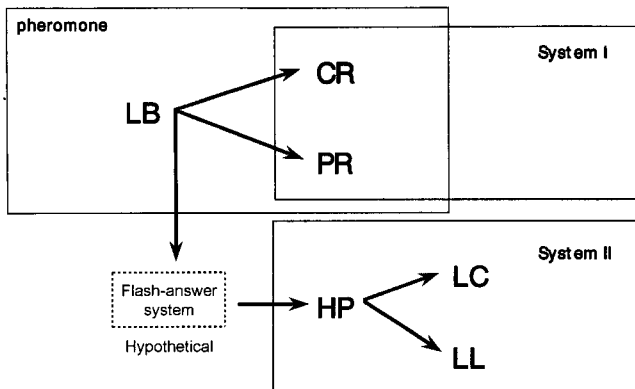


Fig. 3. Diagram of evolutionary divergence of firefly mating systems (Suzuki, 1997).

The process of divergence of the six mating systems is summarized in Fig. 3.

Ecological types

Ecological situations in relation to the flashing time of mate-seeking males have been reported in some Japanese species. Since the flash pattern is strictly related to mating approach, the differentiation of flashing time must function as an important factor of reproductive isolation and speciation. In this viewpoint, we surveyed genetic and geographic differentiation among the ecological types.

Luciola (Hotaria) parvula

Luciola (Hotaria) parvula is distributed in the three major islands of Japan (Honshu, Shikoku, and Kyushu), but size dimorphism (large and small types) was recognized in total body length of males at Mt. Hakone, Kanagawa Prefecture (Ohba, 1986). They were about 10 and 5 mm, and that corresponded to the difference of interflash interval of mate-seeking males about 1 and 0.5 sec, respectively (Ohba *et al.*, 1995; Ohba, 2000). Furthermore, the two types were distributed allopatrically (Ohba, 2000). The large type is distributed in almost all the areas mentioned above, whereas the small type occurs in some parts of Kyushu, Shikoku, and the western area of Honshu.

In order to evaluate genetic differentiation between the two types, Suzuki *et al.* (1993) analyzed allozymes at 17 loci in 10 populations. *L. (H.) tsushimana* from Tsushima Island, a species closely related to *L. (H.) parvula* was used as an outgroup. The phylogenetic tree for these populations constructed by Nei's (1972) genetic distance showed that *L. (H.) parvula* was divided into two groups corresponding to the small and large types (Fig. 4). All the small type populations are clustered within a slightly dif-

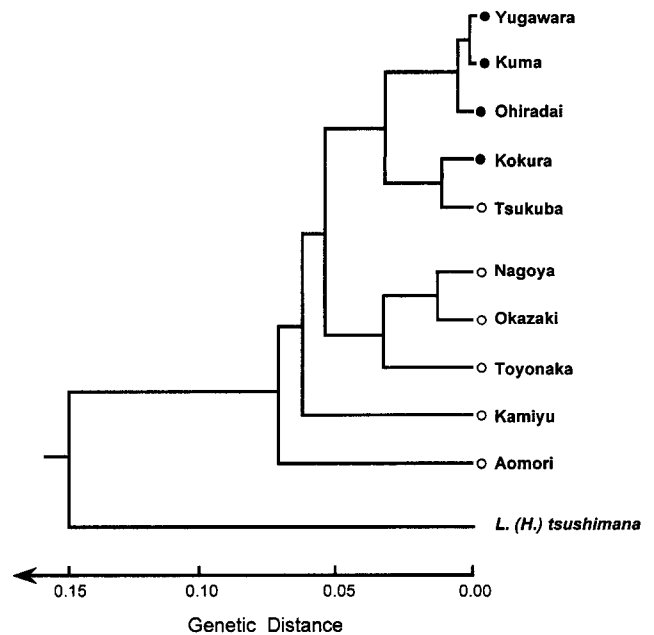


Fig. 4. A phylogenetic tree of 10 populations of *Luciola (Hotaria) parvula* and one population of *L. (H.) tsushimana* constructed from Nei's genetic distance by UPGMA. Solid and open circles at the branch ends indicate small and large types, respectively (Suzuki *et al.*, 1993).

ferentiated group, while the large type populations are considerably differentiated from one another. The genetic distance between the two types was 0.11, and that between *L. (H.) parvula* and *L. (H.) tsushimana* is much higher (0.31) than that within either the large type or small type populations. These findings suggest that the small type originated from an ancestor similar to the large type of *L. (H.) parvula*.

Luciola lateralis

Luciola lateralis is widely distributed throughout the Siberia, Korean Peninsula, and Japan (Hokkaido, Honshu, Shikoku, and Kyushu). Ohba *et al.* (1993) reported ecological differences of this species between Hokkaido and Honshu populations in Japan. Namely, interflash interval of mate-seeking males in Hokkaido is longer (about 1 sec) than that in Honshu (about 0.5 sec), and it takes more than two years for emergence of adult firefly in Hokkaido, whereas it takes one year in Honshu. As the temperature in Hokkaido is too low for the firefly to grow up within a year, the ecological differences are considered as adaptation to cold climate.

Figure 5 shows random amplified polymorphic DNA (RAPD) patterns obtained from genomic DNA of *L. lateralis* in Japan using single primers, OPA-1 and 19. Molecular size of the PCR (polymerase chain reaction)

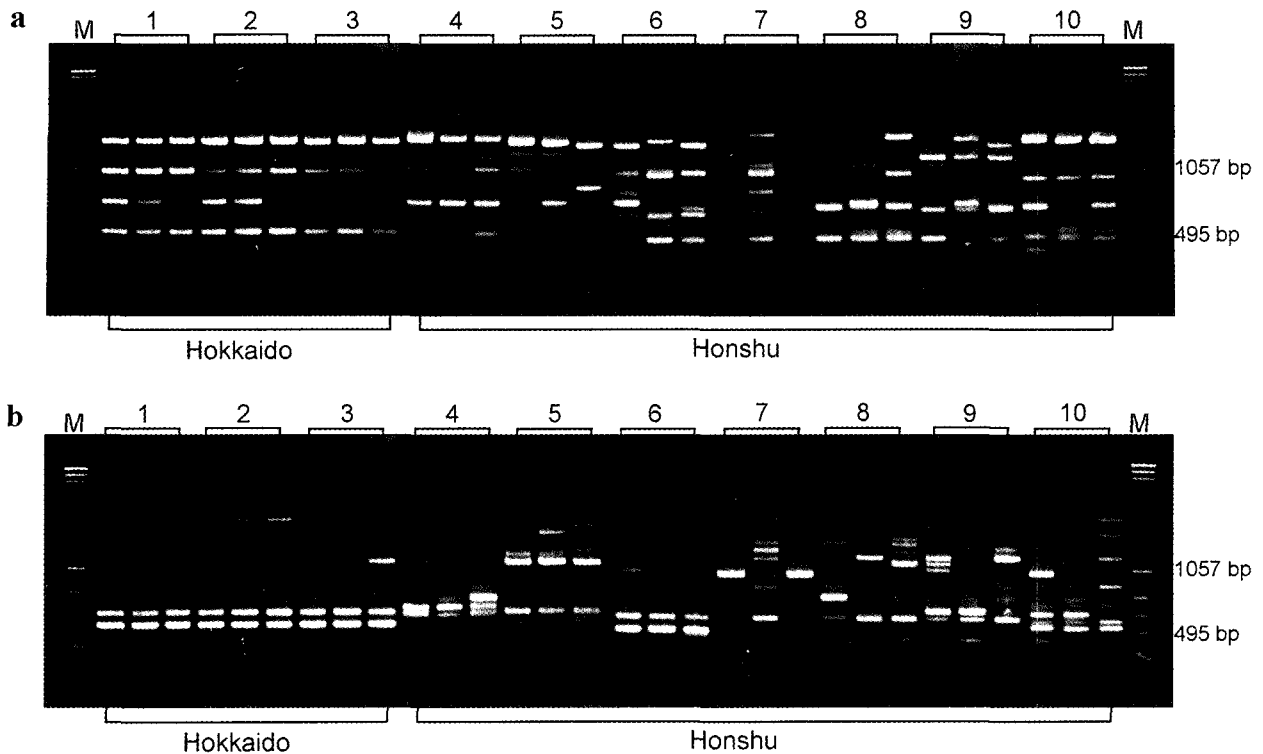


Fig. 5. RAPD patterns obtained from genomic DNA of *Luciola lateralis* in 10 populations (1, Akkeshi; 2, Kushiro; 3, Sapporo; 4, Hirosaki; 5, Morioka; 6, Yonezawa; 7, Ôhara; 8, Koshiji; 9, Iizuna; 10, Miasa). The PCR program consisted of 45 cycles of denaturing at 94°C for 1 min, annealing at 36°C for 1 min, and extension at 72°C for 2 min. The patterns in (a) and (b) were obtained with single primers, OPA-1 (5'ICAGGCCCTTC3') and OPA-19 (5'ICAAACGTCCG3'), respectively.

products in Honshu populations is more variable than that in Hokkaido populations. Phylogeographic survey using mitochondrial DNA is now in progress.

Luciola cruciata

Luciola cruciata is distributed in Japan (Honshu, Shikoku, and Kyushu), but two ecological types have been recognized in the flashing behavior. Namely, interflash interval of mate-seeking males in the east area is about 4 sec (slow-flash type), while that in the west area is about 2 sec (fast-flash type) (Kanda, 1935, 1981). The boundary between the two ecological types nearly corresponds to a great rupture zone called the Fossa Magna which lies from north to south and divides the Honshu into its east and west areas (Ohba, 1984, 1988). Furthermore, in a habitat of the fast-flash type, females aggregate at some sites for oviposition (Kuribayashi, 1979; Yuma and Hori, 1981), whereas in that of the slow-flash type, they do not assemble gregariously for oviposition (Ohba, 1988). These two ecological types are morphologically indistinguishable from each other, although they express the above-described behavioral differences.

Suzuki *et al.* (1996 b) analyzed the degree of genetic differentiation between the two ecological types by using

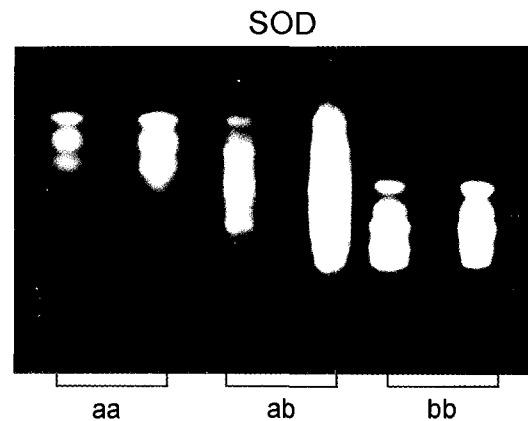


Fig. 6. Electrophoretic pattern of SOD isozymes in *Luciola cruciata*. The aa, ab, and bb are genotypes in the SOD locus estimated from the electromorph.

allozymes at 17 loci in 15 populations, and the genetic distance was 0.09. Figures 6 and 7 show electrophoretic pattern of superoxide dismutase (SOD) allozymes and geographic distributions of the SOD alleles, respectively. The allele *a* is dominant in the west area, while the *b* in the east area. The distribution pattern of the alleles is nearly concordant with that of the ecological types, and

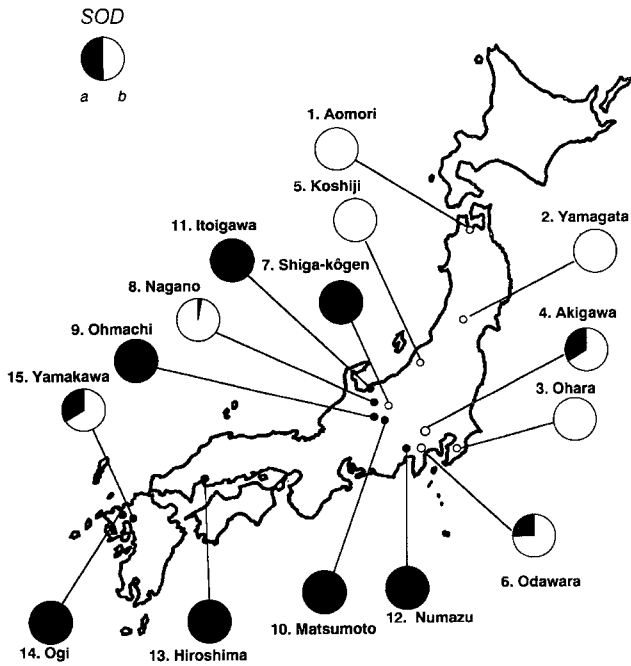


Fig. 7. Allele distribution pattern of the *SOD* locus in *Luciola cruciata*. Circle graphs indicate the percentages of the *a* and *b* alleles. Solid and open circles at the locality position indicate the fast- and slow-flash types, respectively (Suzuki *et al.*, 1996b).

the restriction of gene flow between them is suggested. However, interrelationships among populations within each ecological type and the origin of the two types were not clarified. Recently, Suzuki *et al.* (in preparation) surveyed restriction fragment length polymorphism (RFLP) of the mitochondrial cytochrome oxidase II gene within and among populations of *L. cruciata* collected from 62 sites covering almost all the insect's distribution areas. As a result, 19 haplotypes (A to S) were detected. Based on the nucleotide sequences comparison of the haplotypes, six haplotype-groups (I to VI) were recognized, and their distribution was indigenous to local areas (Fig. 8a). Namely, Group I (containing A and D haplotypes) locates in North Honshu area, Group II (B and C) in Kanto to North Chubu area, Group III (F and G) in Chubu area, Group IV (E, H, I, and J) in West Japan area, Group V (K, L, M, N, O, and S) in North Kyushu area, and Group VI (P, Q, and R) in South Kyushu area. But the boundary between Groups III and IV is overlapping around southwestern part of the Chubu area. Boundaries of the six groups are approximately concordant with geological structures of the Japanese Islands, and a distributional scenario of this species was proposed. The phylogenetic tree of the haplotype-groups (Fig. 9) suggests that the fast-flash type is considered an ancestral form, while the slow-flash type a derived one arisen from the common lineage

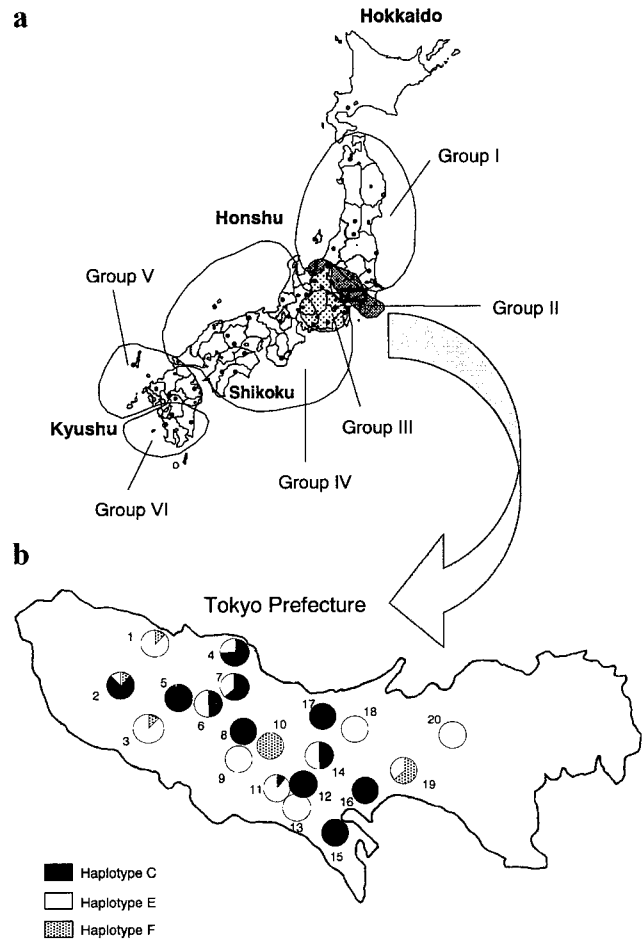


Fig. 8. Distribution map of the *CO II* haplotype-groups of *Luciola cruciata* in Japan (a), and distribution pattern of the haplotypes in Tokyo (b). Circle graphs indicate the percentages of the haplotype C, E, and F, at the localities (Suzuki, 2001).

of Groups I and II. The divergence time between the slow- and fast-flash types is estimated to be about 3.9 to 1.9 Mya (the Pliocene epoch).

Conservation measures

Firefly is occasionally used as a symbolic animal for natural conservation and reconstruction in Japan, because of the beautiful light view displayed by the insect. However, fireflies are easily introduced to other places for tourist attraction, firefly festival, environmental education, and/or personal enjoyment (Watas', 1902; Kanda, 1935, 1981; Minami, 1961, 1983). In particular, introduction is conspicuous in the Japanese popular firefly, *Luciola cruciata*, even though we can recognize such ecological types differentiated genetically. Therefore, there is a possibility that genetic disturbance occurs in this species. According

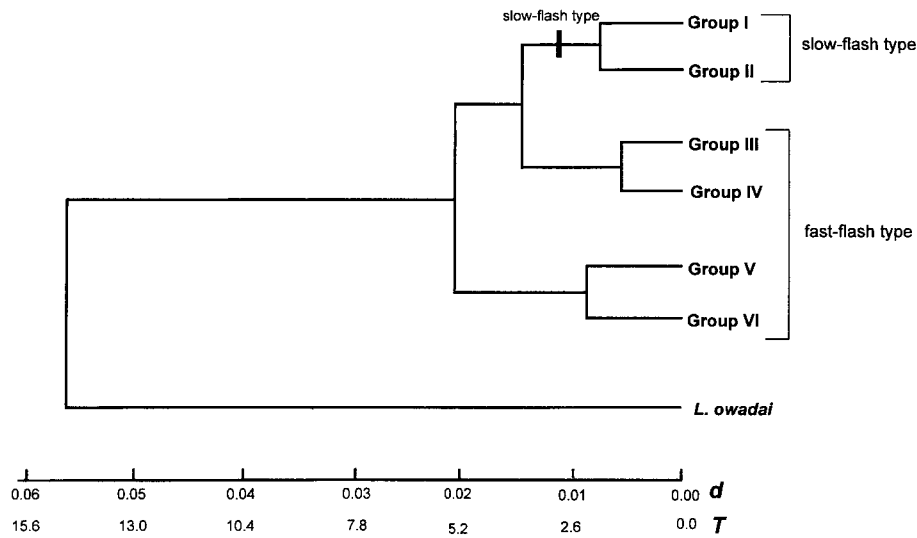


Fig. 9. Phylogenetic tree of the *CO II* haplotype-groups in *Luciola cruciata* constructed by UPGMA with parsimonious allocation of the two ecological types. *d*: evolutionary distance of Kimura's two-parameter model, *T*: divergence time (Mya) (Suzuki *et al.*, in preparation).

Table 2. Haplotypes of *CO II* gene and their frequencies of *Luciola cruciata* in the 20 populations in Tokyo

Population (No. of individuals)	Haplotype (frequency)
1. Sunniwa, Okutama-cho (10)	E(0.90), F(0.10)
2. Kanoto, Hinohara-mura (7)	C(0.72), E(0.14), F(0.14)
3. Senzoku, Hinohara-mura (10)	E(0.90), F(0.10)
4. Nariki, Oume-shi (8)	C(0.75), E(0.25)
5. Irino, Akiruno-shi (8)	C(1.00)
6. Yokosawa, Akiruno-shi (11)	C(0.50), E(0.50)
7. Sugao, Akiruno-shi (7)	C(0.57), E(0.43)
8. Takatsuki, Hachioji-shi (10)	C(1.00)
9. Moto-hachioji, Hachioji-shi (10)	E(1.00)
10. Tangi, Hachioji-shi (4)	F(1.00)
11. Yarimizu, Hachioji-shi (10)	C(0.10), E(0.90)
12. Shimoyugi, Hachioji-shi (2)	C(1.00)
13. Minami-ohsawa, Hachioji-shi (1)	E(1.00)
14. Mogusa, Hino-shi (4)	C(0.50), E(0.50)
15. Onoji, Machida-shi (3)	C(1.00)
16. Sakahama, Inagi-shi (6)	C(1.00)
17. Tamagawa-jôsui, Kodaira-shi (3)	C(1.00)
18. Ogawa, Kodaira-shi (10)	E(1.00)
19. Kojima, Chofu-shi (9)	E(0.44), F(0.56)
20. Kugayama, Suginami-ku (10)	E(1.00)

to the distribution map of the haplotype-groups (Fig. 8a), haplotypes of *COII* gene were surveyed to assess artificial disturbance in 20 populations of Tokyo Prefecture, the Kanto district (Suzuki, 2001).

Haplotypes and their frequencies in the 20 populations are summarized in Table 2. Totally, 3 haplotypes, C, E, and F belonging to the different haplotype-groups, II, IV, and III, respectively are observed. However, there are no geographical associations of the haplotype distribution pattern (Fig. 8b). It has been known that fireflies in the six populations, Sunniwa (site No. 1), Moto-hachioji (9), Tangi (10), Ogawa (18), Kojima (19), and Kugayama (20), were transplanted artificially, and haplotypes of them were E and F. The origins of Ogawa, Kojima, and Kugayama populations are in the distribution area of Group IV, but those of other populations are unknown. In the Sugao (7) population, two haplotypes, C and E, are observed, even though Tokyo is located in Group II area. It has been known that fireflies were transplanted artificially from Group IV area to the original colony. Therefore, coexistence of the heterogeneous haplotypes found in the different haplotype-groups is considered a consequence of the artificial transplantation. The haplotype C is fixed in the Irino (5), Takatsuki (8), Shimoyugi (12), Onoji (15), Sakahama (16), and Tamagawa-jôsui (17) populations, although sample sizes are not so enough. In the other populations, the haplotypes C is observed with the E and F haplotypes except for Minami-ohsawa (13) and Senzoku (3) populations. It is, however, unknown whether artificial transplantation has been done or not in these populations. According to the distribution map of the haplotype-groups, heterogeneous haplotypes from the different haplotype-groups are observed in Tokyo and permeation of artificial disturbance in this area is confirmed at mt DNA level (Fig. 8).

Recently, many problems of biological invasion, e.g. extinction of native species by ecological competitor and genetic disturbance among closely related species, arise in Japan (Nakamura, 1990). Also in firefly fauna, *L. cruciata* is found in Hokkaido, although Hokkaido is not a native distribution area (Environmental Agency ed., 1992). *L. owadai*, the most closely related species to *L. cruciata*, is distributed only in Kume-jima Island. However, *L. cruciata* was caught in Kume-jima Island in 1999. Furthermore, *Pyropyga* species distributed in the North and South America was found in Tokyo, and the possibility of naturalization was pointed out (Goto, 1998). This species is excluded from the list of Japanese firefly in Table 1. Anyway, these are marked cases in artificial introduction.

The artificial transplantation disturbs ecosystem formed by the evolutionary history of the species such as distribution pattern of the haplotype-group in *L. cruciata*. However, it has been done involuntarily by peoples for natural reconstruction. Therefore, natural reconstruction program based on biogeographic and genetic background is urgently needed, and we consider that the genetic diversity studies of populations and data library of the haplotype-groups would contribute to drafting the program. In order to promote natural reconstruction programs to protect genetic resources endemic to the localities, we will propose some policies based on the genetic diversity study, especially for firefly that: 1) To forbid transplantation of fireflies from other places on the biogeographical basis, 2) to protect original colony before transplantation, even if transplantation is required, and 3) if transplantation is required in the case of extinction of the original colony, fireflies from the nearest river system must be transplanted.

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