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ORIGINAL PAPER

# The ecological impact of an introduced population on a native population in the firefly *Luciola cruciata* (Coleoptera: Lampyridae)

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**Abstract** In Japan, Tatsuno Town has been famous for many *Luciola cruciata* fireflies emerging every summer at least since the 1920's. However, in the 1960's, *L. cruciata* fireflies were intentionally introduced from the Lake Biwa area into Matsuo-kyo, the most famous habitat of fireflies in that town. In this study, I examined ambient temperatures and flash rates of *L. cruciata* at four sites including Matsuo-kyo in the Tatsuno area and two sites in the Lake Biwa area. The linear regression of flash rates on temperatures indicated that the Matsuo-kyo population was distinct from the other three populations native to the Tatsuno area, but similar to the two populations native to the Lake Biwa area in terms of flash rates. These results were also supported by a recent molecular biological study, suggesting that the introduced fireflies had a strong ecological impact on the native ones at Matsuo-kyo. The present study emphasizes that we should not transport and release *L. cruciata* fireflies without careful consideration.

**Keywords**: *Luciola cruciata*, Tatsuno Town, introduced fireflies, native fireflies, flash rate, temperature

## Introduction

Fireflies have long attracted the attention of biologists because of their spectacular flashing. However, most studies on fireflies have focused on flash communication, mate competition, and mate choice (Buck and Buck 1966; Lloyd 1966; Carlson et al. 1976; Ohba 2004; Vencl 2004; Lewis et al. 2004; Lewis and Cratsley 2008). Many field

studies on plants and other animals have increasingly noted that native biodiversity is threatened by introduced species or populations (for review, see Wilcove et al. 1998; Ludsin and Wolfe 2001; Lee 2002). Nevertheless, very few studies have examined the cology of introduced fireflies in the context of the conservation of biodiversity (Takeda et al. 2006). In this study, I assess the effect of an introduced population on a native population in the Japanese firefly *Luciola cruciata* (Coleoptera, Lampyridae) and discuss the conservation of native fireflies.

*L. cruciata* is called 'Genji Botaru' in Japan. Of all Japanese firefly species, Japanese people love this firefly best, because males of this firefly hover, emitting spectacular synchronous rhythmic flashes. Therefore, when this firefly is active in early summer, many cities and towns celebrate Firefly Festivals. This firefly has also been considered a national symbol for environmental conservation in Japan, because larvae of this firefly live in non-polluted rivers. Therefore, many local communities have made great efforts to protect this firefly. Recently, Takeda et al. (2006) indicated that the conservation of this firefly would result in the extensive conservation of biodiversity in rural areas. Nevertheless, it is also true that many eggs, larvae, and adults of this firefly have been intentionally transported into new areas. One of the reasons is that people have a strong desire to watch many fireflies flashing every summer in their neighborhoods. Another reason is that local communities or governments want to utilize fireflies in order to arouse public interest in environmental conservation and attract many tourists. However, such intentional introduction may produce serious problems. For example, recent genetic studies on this firefly revealed that intentionally introduced populations caused genetic disturbance to native populations in Tokyo (Suzuki 1997, 2001). However, the ecological impact of introduced fireflies on native ones remains unknown. In this article, I present the impact of introduced fireflies on native ones in Tatsuno Town, Nagano Prefecture, Japan (Fig. 1a).

In Tatsuno Town, there are several sites where many fireflies emerge every summer. Of all these sites, Matsuo-kyo (Fig. 1b) has been best known for a large number of *L. cruciata* emerging every summer at least since 1920's (Katsuno 1968). Therefore, this site was designated as a natural monument by the Nagano Prefectural Government in 1926. After that, however, the number of fireflies gradually decreased because of water pollution. Therefore, people in Tatsuno Town made great efforts to restore the habitat of fireflies and also transported *L. cruciata* fireflies from an area surrounding Lake Biwa into Matsuo-kyo in 1960's (Fig. 1a, b; Iguchi 2003), because the Lake Biwa area was also famous for many fireflies emerging. However, the effect of the introduced fireflies on the native ones was never investigated. Ohba (1988, 2001) found that Matsuo-kyo fireflies synchronously flash at intervals of about 2 s, but he did not know about the introduction of fireflies into this site. Recently, my preliminary study suggested that there might be a similarity in the interflash interval of *L. cruciata* between Matsuo-kyo and the Lake Biwa area (Iguchi 2006). However, no detailed comparison of *L. cruciata* fireflies between these two sites has been carried out, and therefore the similarity in flash is also still ambiguous.

Ohba (1988, 2001, 2004) revealed that the interflash interval of *L. cruciata* exhibits marked geographical variation; that is, the interval is about 2 s in southwestern Japan,

about 3 s in central Japan, and about 4 s in northeastern Japan. Ohba (1988) and Mitsuishi (1990) showed that these geographical differences can be easily revealed by the use of a stop watch, and therefore, many researchers, especially members of the Japan Association for Fireflies Research, investigated interflash intervals of local populations with stop watches at many sites in Japan (for review, see Ohba 1988; Mitsuishi 1990). However, most of these studies have classified interflash intervals without consideration of the effect of temperature.

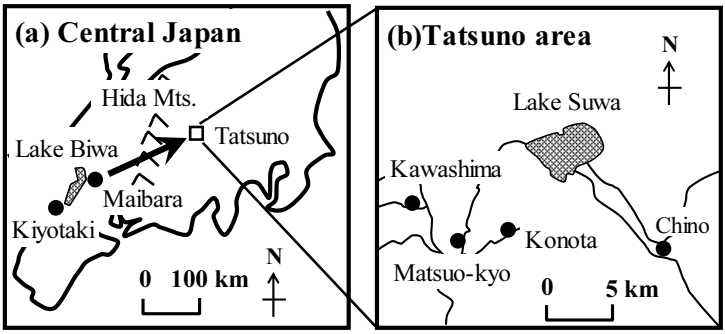


**Fig. 1** **a** Locations of the Lake Biwa and Tatsuno areas in central Japan; 33–38°N, 135–141°E. These two areas are separated by the Hida Mountains. The *arrow* indicates that fireflies were intentionally introduced from Maibara or its vicinity into Matsuo-kyo in the Tatsuno area. **b** Detailed map of rivers and study sites in the Tatsuno area; 35.9–36.1°N, 137.9–138.1°E. Data were collected from Kiyotaki and Maibara in (**a**), and Kawashima, Matsuo-kyo, Konota, and Chino in (**b**).

Ohba (2001) suggested that the interflash interval of *L. cruciata* does not depend on temperature, but he did not carry out any statistical analysis. In contrast, several studies have statistically indicated that the interflash interval of *L. cruciata* largely depends on ambient temperature (Sasai 1999; Iguchi 2002). In addition, Sasai (1999) suggested that the number of flashes per second, namely the inverse of interflash interval (= 1/ interflash interval), might be more useful for exploring geographical variation in flashes of *L. cruciata*. Similarly, Lloyd (2000) found that in *Photinus collustrans* the regression of the inverse of interflash interval on temperature produced a straight line, and he named the inverse of interflash interval 'flash rate'.

In this study, I begin by collecting data on ambient temperatures and flash rates of *L. cruciata* at six sites in the Lake Biwa and Tatsuno areas including Matsuo-kyo. Then, I compare flash rates by regression analysis. I also examine DNA data on this firefly in these two areas. Lastly, I assess the effect of introduced fireflies on native fireflies at Matsuo-kyo. I also emphasize the importance of measuring ambient temperature when investigating geographical variation in flashes of *L. cruciata*.

## Materials and methods

**Table 1** Study sites and researchers

| Area | Site | Coordinate | Researcher |
|---|---|---|---|
| Tatsuno | Matsuo-kyo | 35.9921°N, 138.0014°E | The author, Ohba 2001, Hiyori et al. 2007 |
| | Konota | 35.9942°N, 138.0353°E | The author, Hiyori et al. 2007 |
| | Kawashima | 36.0149°N, 137.9630°E | The author |
| | Chino | 35.9822°N, 138.1602°E | Ohba 2001 |
| Lake Biwa | Maibara | 35.3448°N, 136.3633°E | Kubunden 1997, Hiyori et al. 2007 |
| | Kiyotaki | 35.0417°N, 135.6577°E | Ohba 2001 |

*Study sites*

I collected data on ambient temperatures and flash rates of *L. cruciata* in the Lake Biwa and Tatsuno areas not only by my observations but also from the data of Kubunden (1997) and Ohba (2001). In addition, I obtained mitochondrial DNA data on this firefly from Hiyori et al. (2007). These researchers and their study sites are listed in Table 1. The sites are also shown in Figs. 1a and b. The Lake Biwa and Tatsuno areas are roughly separated by the Hida Mountains (Fig. 1a).

*Measurement of flash rates*

First of all, I confirmed that several groups of more than three fireflies were synchronously flashing here and there at all my observation sites. Each group continuously repeated synchronous flashing. Then, I observed the synchronous flashing of a group of fireflies hovering and flashing within approximately 3–5 m in radius. It is very difficult to measure the flash rate (the number of flashes per second) of *L. cruciata* directly. Therefore, following the method of Sasai (1999), I first measured an interflash interval as the time between the beginning of a synchronous flash and that of the next one to the nearest 0.01 seconds with a digital stop watch (Maruman Maow Sports Timer, Maruman, Japan) between 2100 and 2400 h. This procedure was repeated 5–30 times (on average, 27.4 times) within 30 minutes per night, and each interflash interval obtained was transformed into its inverse (= 1/ interflash interval), namely flash rate. Finally, the mean flash rate was determined each night.

Ambient air temperature was also recorded with an alcohol thermometer (Sato Keiryouki Mfg., Japan; accuracy, ±1 °C) each night to the nearest 0.1 °C at a height at which many fireflies were hovering and flashing. The thermometer was hung on a branch of a tree 30 minutes before each observation to stabilize its reading. In each observation, air temperature did not change more than approximately 1 °C for 30 minutes. Nevertheless, I used the value of temperature measured 15 minutes after the

beginning of each observation.

As mentioned above, I also used the data of Kubunden (1997) and Ohba (2001) (Table 1). Kubunden (1997) measured interflash intervals with a stop watch, and Ohba (2001) did with an oscilloscope.

*Statistical analysis of flash rates*

The data that Kubunden (1997) and I obtained at Matsuo-kyo, Konota, Kawashima, and Maibara were used for regression analysis. As indicated by Sasai (1999), I confirmed strong positive correlations between ambient temperature and mean flash rate in all these four sites (Fig. 2; Pearson correlation, Konota: $r = 0.99$, $n = 7$; Kawashima: $r = 0.88$, $n = 5$; Maibara: r = 0.83, $n = 7$; Matsuo-kyo: $r = 0.98$, $n = 7$; $p < 0.05$ for all). The standard error of each mean flash rate was < 0.007 and less than approximately 1 % of the mean flash rate. Therefore, each standard error was too small to be shown in the graph (Fig. 2). This result suggests that the estimation of each mean flash rate was very precise.

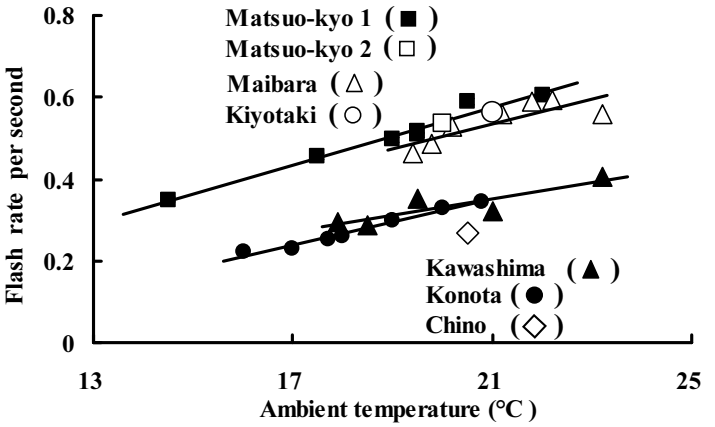


**Fig. 2** Relationship between ambient air temperatures and flash rates of seven *L. cruciata* populations. Mean flash rates are shown, but standard errors were too small (< 0.007) to be shown. *Regression lines* are shown for Matsuo-kyo 1, Maibara, Kawashima, and Konota. Only data points are shown for Matsuo-kyo 2, Kiyotaki, and Chino, because the data at these sites were obtained at a single temperature. Matsuo-kyo 1 and 2 represent the populations observed at Matsuo-kyo by the author and Ohba (2001), respectively.

I fit separate linear regression lines to the data obtained from each site (Fig. 2). To test for heterogeneity in slope and elevation (e.g., *y*-intercepts or *y* values for a given *x* after fitting a common slope), I used analysis of covariance. Once the slopes or elevations of the four populations were significantly heterogeneous, I used Tukey's multiple comparison tests for differences between each pair of slopes or elevations (Zar 1996).

The data of Ohba (2001) were not included in the above regression analysis, because he measured interflash intervals at a single temperature at each site. Instead, his data were first used for analysis of variance, because they were collected at similar temperatures, 20–21 °C (Fig. 2). Then, they were examined by Tukey's multiple comparison tests.

## Results

As shown in Fig. 2, the regression analysis of the Matsuo-kyo (designated by Matsuo-kyo 1), Konota, Kawashima, and Maibara populations revealed no significant heterogeneity in slope ($F_{3,18}$ = 1.96, $P$ = 0.16), but significant heterogeneity in elevation ($F_{3,21}$ = 142.5, $P$ < 0.0001). Therefore, these regression lines were considered parallel and the common slope was estimated as 0.03.

As a result of Tukey's tests ($k$ = 4, $v$ = 18), Matsuo-kyo differed significantly from Konota and Kawashima in the Tatsuno area ($q$ > 20, $P$ < 0.001 for both), but not from Maibara in the Lake Biwa area ($q$ = 2.72, $P$ = 0.25).

As shown in Fig. 2, the analysis of variance of Ohba's (2001) data revealed a significant difference among Matsuo-kyo (designated by Matsuo-kyo 2), Chino, and Kiyotaki ($F_{2,27}$ = 177.6, $P$ < 0.0001). As a result of Tukey's tests ($k$ = 3, $v$ = 27), Matsuo-kyo differed significantly from Chino in the Tatsuno area ($q$ = 19.3, $P$ < 0.001), but not from Maibara in the Lake Biwa area ($q$ = 1.96, $P$ = 0.36).

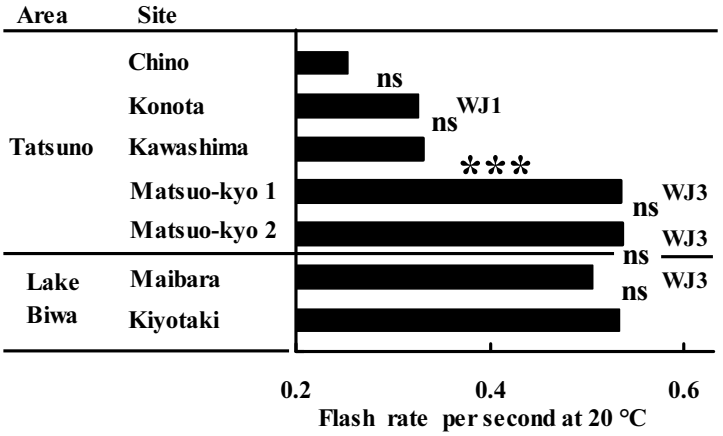


**Fig. 3** Comparison of flash rates among the seven *L. cruciata* populations. The *regression lines* had a common slope, but differed in elevation. Using the common slope, I compared flash rates by Tukey's tests ($k$ = 7, $v$ = 15): *ns*, not significant ($P$ > 0.1); ***, $P$ < 0.001. For convenience, flash rates at 20 °C are shown. WJ1 and WJ3 represent the DNA types of *L. cruciata* identified by Hiyori et al. (2007), which denote Western Japan 1 and 3 types, respectively.

For comparing Ohba's (2001) data with Kubunden's (1997) and my data, I fit the

common slope (= 0.03) of the regression analysis to Ohba's (2001) data points. The results are shown in Fig. 3. In both my data (Matsuo-kyo 1) and Ohba's data (Matsuo-kyo 2), Matsuo-kyo differed significantly from Chino, Konota, and Kawashima in the Tatsuno area, but not from Maibara and Kiyotaki in the Lake Biwa area.

Fig. 3 also shows the DNA data of Hiyori et al. (2007). Matsuo-kyo (Western Japan 3 type, WJ3) was different from Konota (Western Japan 1 type, WJ1) in the Tatsuno area, but identical to Maibara in the Lake Biwa area.

As shown in Fig. 4, WJ3-type fireflies are widely distributed in and around the Lake Biwa area to the west of the Hida Mountains, whereas WJ1-type fireflies are widely distributed in and around the Tatsuno area to the east of the Hida Mountains (Hiyori et al. 2007). However, out of approximately 30 sites surveyed by Hiyori et al. (2007) in and around the Tatsuno area, only Matsuo-kyo belonged to the WJ3 type.

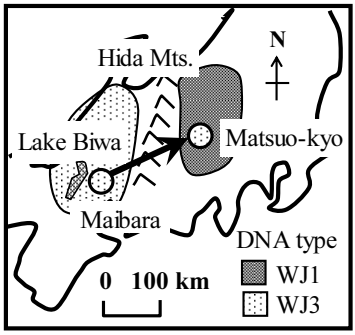


**Fig. 4** Distribution of two DNA types of *L. cruciata*. WJ1 and WJ3 represent the DNA types identified by Hiyori et al. (2007), which denote Western Japan 1 and 3 types, respectively. Except for Matsuo-kyo, the WJ3 type is widely distributed to the west of the Hida Mountains and the WJ1 type to their east. Matsuo-kyo lies to the east of the Hida Mountains, but fireflies there belong to the WJ3-type. The *arrow* indicates that fireflies were intentionally introduced from Maibara or its vicinity into Matsuo-kyo.

## Discussion

As mentioned before, Ohba (1988, 2004) found that *L. cruciata* exhibits marked geographical variation in interflash intervals. However, the present results clearly showed that such geographical variation was affected by ambient temperature. Therefore, when discussing geographical variation in flashes of *L. cruciata*, we should examine the regression of flash rate or interval on ambient temperature rather than flash rate or interval only.

The present study examined temperature-dependent flash rates of *L. cruciata* at six sites in central Japan. Matsuo-kyo, Chino, Konota, and Kawashima lie within a 10-km

radius in the Tatsuno area to the east of the Hida Mountains. However, Matsuo-kyo fireflies were different in flash rate from the other fireflies in the Tatsuno area, but very similar to fireflies in the Lake Biwa area to the west of the Hida Mountains. Both Kubunden (1997) and I measured interflash intervals with a stop watch. However, the data that Ohba (2001) obtained with an oscilloscope supported my observations. The DNA data of Hiyori et al. (2007) also supported my observations. Unfortunately, there are no data on what type of *L. cruciata* existed at Matsuo-kyo before introducing non-native *L. cruciata*. However, it is highly likely that Matsuo-kyo fireflies before the introduction were very similar to Konota and Kawashima fireflies native to the Tatsuno area and not to Maibara and Kiyotaki fireflies native to the Lake Biwa area, because Matsuo-kyo is only 3 or 4 km away from Konota and Kawashima (Fig. 1b), but approximately 160–240 km away from Maibara and Kiyotaki (Fig. 1a). These results suggest that fireflies introduced from Maibara or its vicinity have become successfully established at Matsuo-kyo for about 40 years. A recent study on *L. cruciata* suggests that geographic variation in flash interval can cause pre-mating isolation (Tamura et al. 2005). Therefore, the present results suggest that the introduced fireflies had a strong ecological impact on the native fireflies. The introduced fireflies may have driven the natives away from Matsuo-kyo or driven them to extinction. Otherwise, as a result of hybridization between the native and introduced fireflies, the Matsuo-kyo population may have exhibited the same flash pattern as the Maibara and Kiyotaki populations.

Although *L. cruciata* fireflies geographically vary in their interflash interval, they are not morphologically distinguishable (Suzuki 2001; Ohba 2004). However, even if we can find no morphological difference between native and non-native fireflies, we should avoid the introduction of non-natives into the habitats of natives without careful consideration. Otherwise, the introduction of non-native fireflies may lead to a reduction in native firefly populations, as suggested by the present results.

At Matsuo-kyo, the introduced *L. cruciata* fireflies and their offspring have been raised in the field by the Tatsuno Town Government. However, the Tatsuno Town Government has taken no measures to prevent the spread of these fireflies. Matsuo-kyo is located near the source of the Tenryu River (approximately 210 km in length), one of the longest rivers in Japan. Therefore, it is likely that the offspring of the introduced fireflies will spread to new areas along the Tenryu River, especially because larvae of this species are aquatic. Several natural habitats of this species exist below Matsuo-kyo (Mitsuishi 1990). Therefore, such natural habitats may be affected in future by the offspring of the introduced fireflies. Further ecological and genetic research is urgently required to reveal the distribution of the non-native fireflies and protect the native fireflies.

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