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Temperature-dependent geographic variation in the flashes of the firefly *Luciola cruciata* (Coleoptera: Lampyridae)

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This study examined the regression of male interflash intervals of *Luciola cruciata* on ambient air temperatures at five sites in central Japan. The result indicated a significant negative correlation between these two variables at any of the sites. The regression lines varied from site to site, and therefore the five local populations were classified into three types; the fast-flash, slow-flash, and intermediate types. This classification was supported by molecular biological studies, but contradicted the results of previous ecological studies based on interflash intervals only. My result suggests that first the slow-flash type and then the intermediate type evolved from an ancestral fast-flash type. The geographic variation in flashes of this firefly may have arisen from shifts in the response of male flashes to ambient temperature.

Keywords: *Luciola cruciata*; interflash interval; ambient temperature; geographic variation

Introduction

Fireflies generally have species-specific flash patterns to communicate with each other (Lloyd 1966, 1973; Ohba 1983, 2004; Lewis and Cratsley 2008). However, it remains unclear how these flash patterns have evolved. Some firefly species are well known for their spectacular synchronous flashing (Buck and Buck 1966; Lloyd 1973). The Japanese firefly *Luciola cruciata* (Coleoptera, Lampyridae) is a particularly well-studied species whose males show mass synchronous flashing (Ohba 1983, 2004). The most interesting feature of the synchronous flashing of this species is that its interflash interval varies geographically from about 2 s in western Japan to about 4 s in eastern Japan (Kanda 1935; Ohba 1988, 2001, 2004; Tamura et al. 2005). Ohba (1988) and Mitsuishi (1990) showed that these geographic differences can be easily detected with a stop watch. However, no detailed statistical analysis of these differences has ever been performed except for an experimental study by Tamura et al. (2005). My recent field study (Iguchi 2009) demonstrated

that even when *L. cruciata* fireflies were transported into other areas, they still showed their original interflash intervals. Molecular biological studies have indicated that these geographic differences arose from genetic differences (Suzuki 1997, 2001; Yoshikawa et al. 2001; Suzuki et al. 2002). Nevertheless, they are not morphologically distinguishable (Suzuki 2001; Ohba 2004). On the other hand, recent ecological studies have indicated that the interflash intervals of *L. cruciata* largely depend on ambient air temperature (Iguchi 2002; Abe et al. 2004). Similar temperature dependence of firefly flashes has also been observed in other firefly species (Lloyd 1966, 2000; Carlson et al. 1976; Michaelidis et al. 2006). Abe et al. (2004) predicted that the geographic variation observed in *L. cruciata* would be artifacts resulting from differences in ambient temperature, and that if interflash intervals were measured at a constant temperature, no geographic variation would be observed. However, this prediction has not yet been tested in detail in the field.

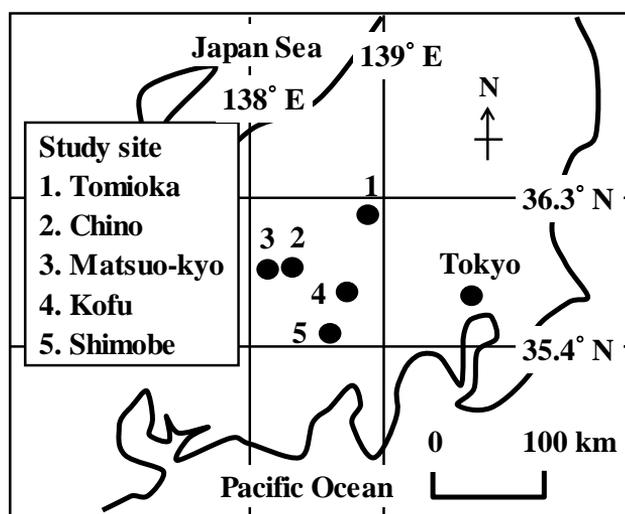


Figure 1. Locations of the five study sites, Tomioka, Chino, Matsuo-kyo, Kofu, and Shimobe in central Japan. For convenience, the location of Tokyo, the capital of Japan, is also shown.

In the present paper, I test the prediction of Abe et al. (2004) by exploring the relationship between ambient temperatures and interflash intervals at five sites in central Japan; namely, Tomioka, Chino, Matsuo-kyo, Kofu, and Shimobe (Figure 1). *L. cruciata* populations in central Japan have been well studied both ecologically (Ohba 1988, 2001; Mitsuishi 1990) and genetically (Yoshikawa et al. 2001; Suzuki et al. 2002; Baba et al. 2005; Hiyori et al. 2007), because this region corresponds to the boundary between the eastern and western flash types. The ecological studies explored interflash intervals only and classified the five populations into two groups; Matsuo-kyo (the western 2-s flash type) and the other four (the eastern 4-s flash

type) (Kanda 1935; Ohba 1988; Suzuki et al. 2002). However, the molecular biological studies based on DNA haplotypes classified them into three groups; namely, Tomioka, Matsuo-kyo and the other three (Yoshikawa et al. 2001; Suzuki et al. 2002; Baba et al. 2005; Hiyori et al. 2007). This discrepancy between the ecological and molecular biological studies remains unexplored.

In this study, I investigated interflash intervals of this firefly and ambient temperatures at the above five sites. Then, I conducted a regression analysis of these two variables. The result suggests that the five regression lines corresponding to the five *L. cruciata* populations reflect true geographic variation in the interflash intervals of this species. The aim of this paper is to show the importance of such regression analysis. The result is also discussed in the context of the evolution of temperature- dependent flash patterns in this species.

Materials and methods

Study sites

The five study sites are located in central Japan and 50 to 100 km away from each other (Figure 1). The location data (latitude and longitude coordinates) are shown in Table 1.

Measurement of interflash intervals

Observations were carried out on 4–6 nights at each site between May and July in 2001 and 2003. In *L. cruciata*, while searching for females, many males are hovering and flashing synchronously. Therefore, I observed the synchronous flashing of a group of approximately 3–20 fireflies hovering within approximately 3–5 m in radius between 21:00 and 24:00. Then, I paid attention to a single firefly in the group flashing synchronously and then measured its interflash interval as the time between the beginning of a flash and that of the next one to the nearest 0.01 seconds with a digital stop watch (Maruman Maow Sports Timer, Maruman, Japan; accuracy, 0.01s). My recent field study (Iguchi 2009) demonstrated that the average of about 30 measurements with a stop watch did not differ significantly from that of measurements with an oscilloscope. Therefore, this procedure was repeated 30 times within 30 minutes per night.

Table 1. Study sites.

| Sites | Coordinates |
|------------|-----------------------|
| Tomioka | 36.2711°N, 138.8103°E |
| Chino | 35.9837°N, 138.1940°E |
| Kofu | 35.6911°N, 138.5741°E |
| Shimobe | 35.4464°N, 138.4592°E |
| Matsuo-kyo | 35.9921°N, 138.0014°E |

I also recorded ambient air temperature to the nearest 0.1 °C with a thermometer (Sato Keiryoku Mfg., Japan; accuracy, ± 1 °C) hanging from a branch of a tree at a height at which the fireflies were hovering and flashing. The thermometer was set up 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 first measurement of interflash intervals.

Data analysis

First, I calculated a linear regression of interflash intervals on ambient temperatures at each of the five sites. Then, I used analysis of covariance to test for heterogeneity in slope and elevation (that is, y values for a given x after fitting a common slope). Once the slopes or elevations of the five populations were significantly heterogeneous, I used Tukey's multiple comparison tests for differences between each pair of slopes or elevations (Zar 1996).

Results

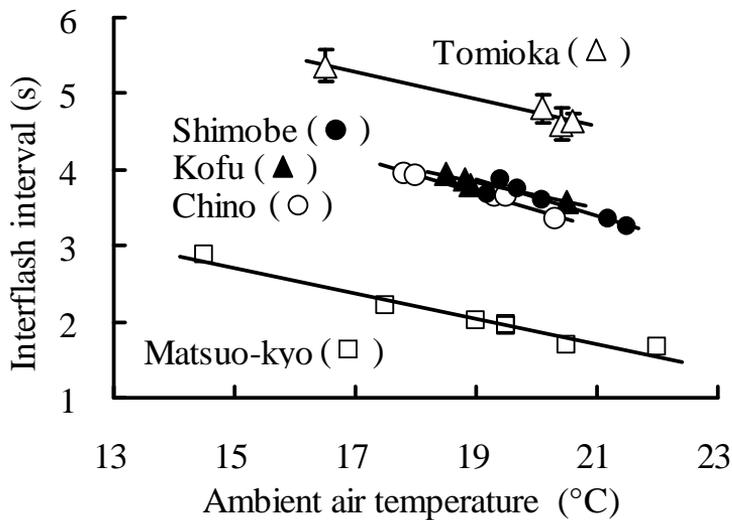


Figure 2. Linear regressions of interflash intervals on ambient air temperatures at the five study sites. Mean interflash intervals and their standard errors are given. However, the standard errors at Shimobe, Kofu, Chino, and Matsuo-kyo were too small (< 0.06) to be shown.

There was a significant negative correlation between ambient temperatures and interflash intervals at any of the five sites (Figure 2; Pearson correlation, Tomioka: $r = -0.30$, $n = 120$; Chino: $r = -0.70$, $n = 150$; Matsuo-kyo: $r = -0.80$, $n = 180$; Kofu: $r =$

= -0.75, $n = 120$; Shimobe: $r = -0.80$, $n = 180$; $p < 0.001$ for all). The analysis of covariance indicated no significant heterogeneity in slope among the five populations ($F_{4,770} = 1.39$, $P = 0.24$), but significant heterogeneity in elevation ($F_{4,774} = 1081.7$, $P < 0.001$). Tukey's multiple comparison tests ($k = 4$, $v = 562$) indicated no significant difference in elevation among Chino, Kofu, and Shimobe ($q < 3.75$, $P > 0.05$ for any combination of two sites), but significant differences between each of the three sites and Tomioka ($q > 29.5$, $P < 0.001$ for any combination) and between each of the three sites and Matsuo-kyo ($q > 54.0$, $P < 0.001$ for any combination). Consequently, the five populations were classified into three types with regard to interflash intervals; Tomioka (slow-flash type), Matsuo-kyo (fast-flash type), and the other three sites (intermediate-flash type).

Discussion

It is noteworthy that the above three types of *L. cruciata* populations differed in interflash interval from each other at any temperature measured. Abe et al. (2004) predicted that if interflash intervals were measured at a constant temperature, they would not vary geographically. However, my result clearly contradicts their prediction. Several studies have shown the temperature dependence of interflash intervals not only in *L. cruciata* but also in other firefly species (Lloyd 1966, 2000; Carlson et al. 1976). However, the present study is the first to show how ambient temperature contributes geographic variation in interflash intervals. Molecular biological studies on this firefly showed that this geographic variation is the result of genetic variation (Yoshikawa et al. 2001; Suzuki et al. 2002). Moreover, Tamura et al. (2005) experimentally showed that this geographic variation reflects variation in male preference for interflash intervals. My result suggests that this geographic variation may have evolved through shifts in the response of male flashes to ambient temperature.

With regard to the five populations examined here, previous ecological studies examined interflash intervals only and classified them into two groups; namely, Matsuo-kyo and the other four (Kanda 1935; Ohba 1988; Suzuki et al. 2002). However, my result clearly classified them into three groups; namely, Tomioka, Matsuo-kyo and the other three. My result was also supported by molecular biological studies (Yoshikawa et al. 2001; Suzuki et al. 2002; Baba et al. 2005; Hiyori et al. 2007), suggesting that the regression of interflash intervals on ambient temperatures better explains geographic variation in *L. cruciata*.

Molecular biological studies (Yoshikawa et al. 2001; Suzuki et al. 2002) have shown that the fast-flash type is an ancestral flash type and that the slow-flash type evolved from an ancestral fast-flash type. However, the phylogenetic position of the intermediate type was unclear. As shown in Figure 3, my result suggests that first the slow-flash type and then the intermediate type evolved from an ancestral fast-flash type.

Ohba's pioneer studies first revealed distinct geographic variation in the interflash intervals of *L. cruciata* (Ohba 1988, 2001, 2004). Many ecological and molecular biological studies on this species have been based on his studies (Suzuki

1997, 2001; Yoshikawa et al. 2001; Suzuki et al. 2002; Tamura et al. 2005; for a review, see Lewis and Cratsley 2008). Ohba (2001) also found a negative correlation between temperature and interflash intervals at a single study site. However, he did not consider the effects of temperature on interflash intervals at many other sites when classifying local populations of *L. cruciata* as the 2-s, 3-s, and 4-s flash types (Ohba 2001, 2004). My result showed that interflash intervals of this species varied from site to site, depending on temperature. Therefore, it may be necessary to reexamine both interflash intervals and ambient temperatures throughout Japan in order to reveal true geographic variation in *L. cruciata*.

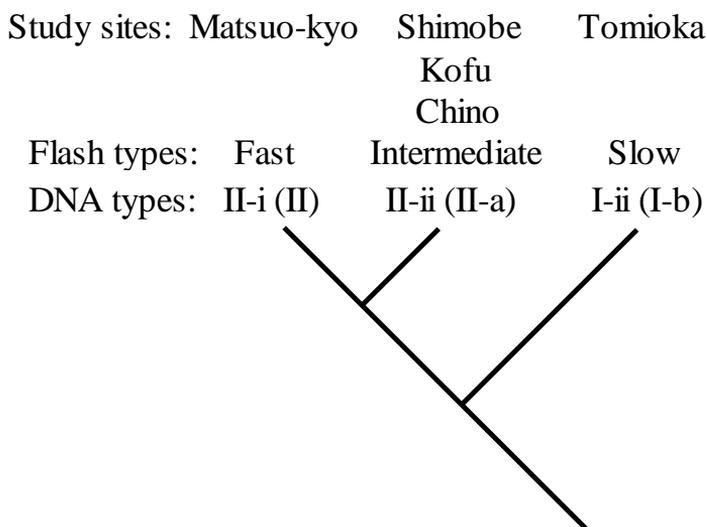


Figure 3. The relationship between flash types and DNA types. The phylogenetic tree was constructed on the basis of mitochondrial DNA studies (Yoshikawa et al. 2001; Suzuki et al. 2002). The three flash types are shown in Figure 2. In the DNA types, II-i, II-ii, and I-ii denote Suzuki et al.'s (2002) classification, and II, II-a, and I-b denote Yoshikawa et al.'s (2001) classification.

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