

Relationship between Morphology and Phylogeny in the Slow-Flashing Genji Firefly, *Nipponoluciola cruciata*, from Shizuoka Prefecture, Central Japan

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Abstract

The Genji firefly, *Nipponoluciola cruciata*, exhibits geographic variation in flash behavior, morphology, and phylogeny across Japan. Although populations in Shizuoka Prefecture belong to the western phylogenetic lineage, they display the slow-flash pattern characteristic of eastern populations. To examine the relationship between morphology and phylogeny in this unusual population, published morphological data were reanalyzed using principal component analysis (PCA). Male specimens from Gunma, Yamanashi, and Shizuoka Prefectures were compared based on external and genital morphological characters. The PCA of genital morphology clearly separated the Shizuoka and Gunma populations, whereas it indicated substantial similarity between the Shizuoka and phylogenetically related Yamanashi populations. In contrast, the PCA of external morphology suggested greater similarity between the Shizuoka and Gunma populations than between the Shizuoka and Yamanashi populations. These results suggest that the Shizuoka population exhibits different patterns of morphological affinity depending on the trait category. Together with its previously reported slow-flash pattern, these findings highlight the distinctiveness of the Shizuoka population and suggest that morphological variation in *N. cruciata* does not correspond uniformly with phylogenetic relationships.

Keywords

Genji Firefly, PCA, Morphology, Phylogeny, Shizuoka

1. Introduction

The aquatic firefly *Nipponoluciola cruciata* (formerly *Luciola cruciata*; Coleop-

tera: Lampyridae), commonly known as Genji-botaru (the Genji firefly), is endemic to Japan and occurs throughout the country's major islands except Hokkaido. One of Japan's most familiar insects [1], the Genji firefly exhibits geographic variation in male flash intervals, ranging from approximately 2 s in western Japan (fast-flash type) to 4 s in eastern Japan (slow-flash type) [2]-[4]. Consistent with this behavioral differentiation, molecular phylogenetic studies have revealed clear genetic differentiation between the two lineages [5]-[8].

Although interflash intervals are temperature-dependent [9] [10], the geographic transition zone between the fast- and slow-flash types remains closely aligned with the molecular phylogenetic boundary identified by the studies mentioned above, which corresponds approximately to the Kanto Mountains, even after adjusting for temperature [10] [11]. Previous studies suggested that these two flash types diverged approximately 11 Ma during the Miocene in association with the uplift of the Kanto Mountains [12]. Recent studies have further revealed geographic variation in body shape, with individuals from western Japan exhibiting broader bodies than those from eastern Japan [13]-[15].

Despite the general concordance between flash behavior and molecular phylogenetic patterns, notable exceptions exist. For example, Shizuoka Prefecture lies west of the Kanto Mountains (Figure 1) and is inhabited by Genji fireflies belonging to the western phylogenetic lineage; however, they exhibit the slow-flash pattern characteristic of eastern populations [16].

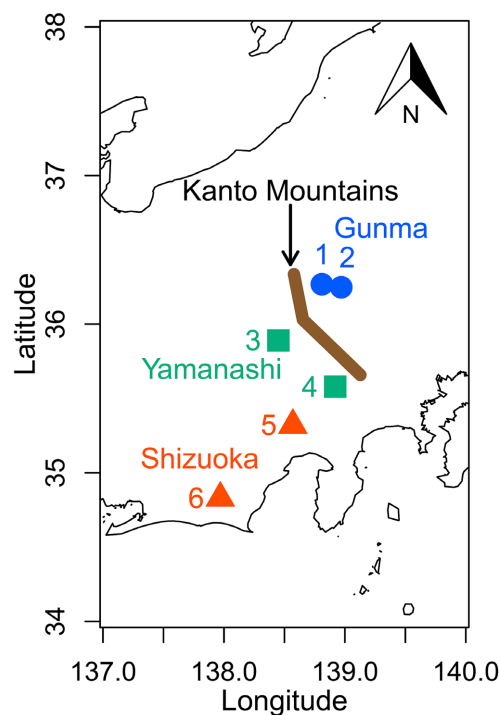


Figure 1. Map of the study localities in Gunma, Yamanashi, and Shizuoka Prefectures, central Japan. Numbers denote the following localities for specimens used in this study: 1. Tomioka, Gunma; 2. Yoshii, Gunma; 3. Takane, Yamanashi; 4. Tsuru, Yamanashi; 5. Fujinomiya, Shizuoka; 6. Kakegawa, Shizuoka.

My previous work [15] identified male body-shape differences between populations east and west of the Kanto Mountains based on datasets from Imasaka [13] [14]. Building on these findings, the present study reanalyzes these datasets to compare male body-shape variation among populations in Gunma, Yamanashi, and Shizuoka Prefectures (Figure 1). The aim of this study is to examine the relationships among phylogeny, morphological variation, and flash patterns in male Genji fireflies from these prefectures.

2. Materials and Methods

Morphological measurements of male Genji fireflies were extracted from Table 2 of Imasaka [13] and Table 2 of Imasaka [14]. Specimens were collected from two localities in Gunma Prefecture, two in Yamanashi Prefecture, and two in Shizuoka Prefecture, central Japan (Figure 1). The sample size for each locality is shown in Table 1.

Table 1. Sample size for each locality.

Locality	Sample size
Tomioka, Gunma prefecture	5
Yoshii, Gunma prefecture	5
Takane, Yamanashi prefecture	6
Tsuru, Yamanashi prefecture	5
Fujinomiya, Shizuoka prefecture	6
Takegawa, Shizuoka prefecture	4

Measured characters included total body length, elytral width (width across the basal portion of the elytra), and six male genital characters of the aedeagus, following the definitions illustrated in Plate 2 (p. 12) of Imasaka (2010): g, difference in length between the median lobe and the left lateral lobe; h, thickness of the apical portion of the median lobe; j, thickness of the basal portion of the left lateral lobe; k, length of the left lateral lobe from base to apex; m, length of the apical portion of the median lobe; and n, width of the middle portion of the median lobe. All measurements were recorded to the nearest 0.1 mm. Prior to analysis, all variables were standardized to a mean of 0 and a standard deviation of 1.

Specimens with missing genital measurements were excluded from analyses of genital morphology. In addition, specimen B82 was excluded because it exhibited an implausibly small value for genital character j (Table 2 in Imasaka, 2012), which was considered a typographical error.

To evaluate morphological variation, principal component analysis (PCA) was conducted using the `prcomp` function in the R package `stats` (R version 4.6.0). PCA plots were generated using the R package `factoextra` (version 2.2.0). To assess the robustness of the results to the exclusion of specimen B82, an additional PCA was also conducted with B82 retained as a sensitivity analysis.

3. Results

In the PCA of genital morphology (**Figure 2**), the Gunma population was separated from the Shizuoka and Yamanashi populations, whereas substantial overlap was observed between the latter two populations. Inclusion of specimen B82 slightly changed the orientation of the PCA axes and the proportion of variance explained by the first two principal components. However, the overall pattern remained similar: the Gunma population was still separated from the Shizuoka and Yamanashi populations, whereas substantial overlap persisted between the latter two populations. Therefore, inclusion of B82 did not materially affect the interpretation of the PCA results.

In the PCA of external morphology (**Figure 3**), the Gunma and Yamanashi populations tended to differ primarily along the second principal component (Dim2), whereas substantial overlap was observed among all three populations along the first principal component (Dim1). Unlike the PCA of genital morphology, clear separation among populations was not evident in the external morphology data. Nevertheless, the Shizuoka specimens formed a relatively compact cluster that overlapped extensively with the Gunma morphospace and showed less overlap with the Yamanashi specimens.

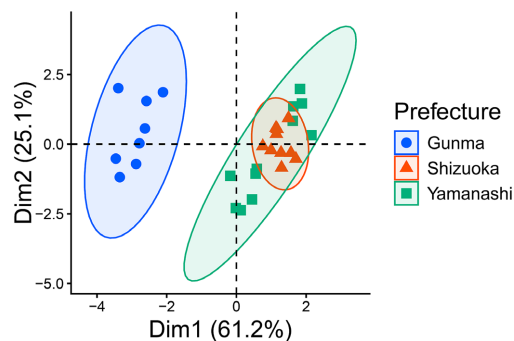


Figure 2. Principal components analysis (PCA) of genital morphology. Individual scores along the first two principal components (Dim1 and Dim2) are color-coded by prefectures (Gunma, Shizuoka, and Yamanashi). Ellipses represent 95% confidence regions.

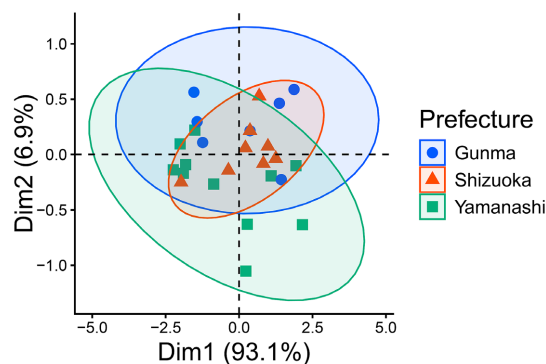


Figure 3. Principal components analysis (PCA) of external morphology. Individual scores along the first two principal components (Dim1 and Dim2) are color-coded by prefectures (Gunma, Shizuoka, and Yamanashi). Ellipses represent 95% confidence regions.

4. Discussion

Taken together, the phylogenetic, morphological, and behavioral data suggest a complex pattern in the Shizuoka population. Although Shizuoka belongs genetically to the western lineage, the morphological and behavioral datasets do not consistently align it with either the Gunma or Yamanashi populations. Genital morphology clearly separates Shizuoka from Gunma, whereas external morphology and flash pattern show greater similarity to Gunma. This incongruence among traits suggests that different phenotypic components may have followed distinct evolutionary trajectories. One possible explanation is historical introgression between eastern and western lineages, which may have contributed to variation in external morphology and flash behavior within the Shizuoka population. Alternatively, similarities in these traits may reflect local adaptation or convergent responses to ecological conditions in Shizuoka. In contrast, genital morphology may have retained characteristics associated with the western lineage. Given the limited ability of the present analyses to discriminate Shizuoka reliably from Gunma or Yamanashi, these interpretations should be regarded as working hypotheses requiring further investigation.

This study has several limitations. First, only two external morphological variables (body length and elytral width) were examined, whereas six genital morphological variables were analyzed. As a result, external morphology may not have been characterized as comprehensively as genital morphology. Second, flash behavior was not recorded from the same specimens used in the morphological analyses. Consequently, comparisons between morphology and flash behavior rely on previously reported prefecture-level patterns rather than direct measurements from the analyzed populations. Future studies incorporating a broader range of external morphological traits together with behavioral observations and genetic data from the same populations will be necessary to provide a more comprehensive understanding of phenotypic variation in *N. cruciata*.

5. Conclusion

This study suggests that phylogeny, morphology, and flash behavior do not necessarily evolve in concert in male Genji fireflies. Although the Shizuoka population belongs genetically to the western lineage, the morphological and behavioral datasets show patterns that are not fully congruent with the phylogenetic relationships. In genital morphology, the Shizuoka population is clearly distinguished from the Gunma population, whereas external morphology and flash pattern show greater similarity to Gunma. Such variation may reflect processes including historical introgression, differences in rates of morphological evolution, or local adaptation, although additional evidence is required to evaluate these alternatives. Overall, our findings highlight the complexity of phenotypic variation in Genji fireflies and the value of integrating genetic, morphological, and behavioral data when examining population divergence. Future studies incorporating genomic data, larger sample sizes, and ecological analyses will be important for clarifying

the evolutionary processes underlying the characteristics observed in the Shizuoka population.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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