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Chapter

Firefly Translocation: A Case Study of Genetic and Behavioral Evaluation in Thailand

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Abstract

Conservation translocation is frequently used to conserve the threatened fauna by releasing individuals from the wild or captive populations into a particular area. This approach, however, is not successful in many cases because the translocated populations could not self-sustain in the new habitats. In this chapter, I reviewed the concept of translocation for conservation and the factors associated with the success rate. I used example problems from several cases involving different insect taxa. With its often high potential to mass rear in captivity, captive breeding can be a powerful tool by assuring large population size for insect translocation, which can result in a high success rate. However, genetic consequences from inbreeding and genetic adaptation to captivity can reduce the fitness of the captive population to establish successfully in the wild. Additionally, as the evidence in Japanese fireflies shows, the genetic differences between the translocated and local populations should be considered for a sustainable translocation program. A case study involved genetic and behavioral evaluation of *S. aquatilis* populations to assess the possibility of including the species for the firefly translocation program in Thailand. Although the results revealed no genetic variation among populations, examination of the variation in flash signals showed that the long-distance population had a longer courtship flash pulse than other populations in the Bangkok Metropolitan Region. With no geographical barrier, the light pollution and urbanization are probably important fragmented barriers causing adaptation of flash communication to increase the fitness. As a consequence, firefly translocation should consider flash variation between populations to prevent this potential pre-mating isolation mechanism from resulting in probable lower translocation success rates.

Keywords: Lampyridae, aquatic firefly, *Sclerotia aquatilis*, flashing behavior, population genetic, intraspecific variation, TiLIA software

1. Introduction

Fireflies have long been attracted the attention of people because of their fascinating flashing communication behavior [1]. In the past, firefly flashes on mangrove trees along the river were used as landmarks for boat navigation in the nighttime; while nowadays firefly habitats become "firefly tour sites" for nighttime activity and for supporting economic benefit to local communities [2]. Unfortunately, firefly populations decrease or disappear from many areas worldwide due to habitat loss from growing of city developments, light pollution, water pollution and pesticide uses, which cause habitat destruction or fragmentation [3–7]. This same situation is faced by other insects [8]. In addition, firefly tourism without proper management could result in decreased firefly populations [2, 9, 10]. The problem has, thus, led to increased public awareness of firefly conservation.

Firefly conservation by reintroduced captive populations into the wild has received much attention. The successful captive breeding of some firefly species has intrigued numerous naturalists and conservationists including tourism stakeholders to plan to introduce captive breeding firefly populations into many areas to create firefly conservation sites, environmental learning centers and firefly tourism spots. The firefly mass rearing has been successful in some aquatic species, including *Aquatica leii* [11], *A. ficta* [12], *A. hydrophila* [13], *A. lateralis* [14], *S. aquatilis* [15, 16], and *S. substriata* [17]. A few of them have been used for conservation translocation. Many parks in Taipei, Taiwan were restored for suitable habitat and captive bred *A. ficta* fireflies were released [18–22]. In Korea, *L. lateralis* habitat (both running water and lentic water areas) was artificially created for releasing the mass reared populations of the species for ecotourism purposes [23]. As a symbol of nature in Japan, many firefly reintroduction and restoration projects of *L. cruciata* and *L. lateralis* have been done over the centuries, but not all of them have been successful [24]. Unfortunately, there are many cases showing strong ecological impact of introduced firefly populations on the native populations, which might eventually lead to the loss of the native populations in Japan [25]. This problem occurs where there is geographical isolation, based on examined differences of flash rate and genetic studies [26]. Therefore, the study of the impact of firefly translocation is essential prior to implementation of the program. Such impact studies have been lacking in Thai firefly translocation projects. Background information on genetic and behavioral variations among populations is necessary for development of a sustainable firefly reintroduction programs.

2. General aspects of translocations for conservation

Conservation translocation (population restoration) or called "ex situ conservation." Under the definition of the IUCN this is the intentional movement of released organisms from one to another site for conservation benefits [27]. That consists of two terms: (i) "reinforcement" which is augmenting a species where it already exists and (ii) "reintroduction" which is returning a species back to where it has disappeared [28]. With the increasing of habitat loss and fragmentation resulting in high species extinction rates and reduction of overall biodiversity, translocation of species may become an important management tool for recovery of the diminished or lost populations.

Many translocation programs have been carried out in many rare, threatened and keystone species to conserve species and genetic diversity. For example, European bison [29], Lake Sturgeon [30], Persian wild ass [31], green and golden bell frog [32], red wolves [33], and a few insects, (i.e., damselfly [34], field cricket [35] and fireflies [25]). Most of them have involved vertebrates, especially mammals and birds [36]. Consequently, translocation became an important conservation technique for birds in New Zealand [37]. However, as mentioned above, little work has been done in insect taxa.

The success of translocations was defined as resulting in self-sustaining populations in the release area. The success rate is affected by many factors. For example, species, habitat quality of the release areas, location of the release point, origin of animals (captivity or wild), food habit (carnivore, herbivore and omnivore), clutch size, population density and competitors [36]. The research analyzed from translocation studies of 134 bird and 64 mammal projects concluded that the keys for high translocation success rate were releasing wild-caught animals, having herbivore food habits, releasing a large density, releasing in excellent quality habitats and releasing at the center of the area. In addition, the reproduction rate and generation length might affect the population sizes, chances of survival and genetic diversity of the target [38].

Many problems of population establishment from translocation were investigated. The small released populations might result in demographic and genetic consequences, for example, inbreeding depression [38]. Moreover, in the cases of releasing of a captive breeding population, the captive-born individuals provided from benign and stable breeding environments frequently have reduced fitness and high extinction rates after release into the wild. The physiological, behavioral and ecological problems from inbreeding depression, mutation accumulation, loss of genetic diversity and genetic adaptation to captivity were considered [39–43]. These could affect success of translocation programs through low adaptive potential to environmental changes [44]. Thus, many recommendations for dealing with the genetic issues are as follow: (i) minimizing numbers of generations in captivity, (ii) maintaining isolated captive populations with different genetic strains to reduce genetic load, (iii) allowing half-sib mating in captivity to reduce genetic adaptation to captivity and preserve genetic variation, (iv) minimizing kinship by equalizing family sizes and crossing, (v) observing the behaviors that might be lost in captivity, (vi) creating a rearing environment similar to the natural habitat to minimize the artificial selection, (vii) evaluating other risks (i.e., diseases), (viii) and collecting and analyzing long-term monitoring data routinely [39, 41–42, 45–47]. Although returning a lost species might not be same as the outcome of ecosystem restoration, the species perform ecosystem functions and generally relate to the other species. Polak and Saltz [48] suggested that the study on the effects of reintroductions on ecosystem functions should be integrated into the programs. Further, an overlooked issue of genetic impact is genetic contamination by maladaptive genotypes from reproductive crossing between genetically differentiated populations. That could push the recipient population toward extinction [49]. Therefore, the introgression with the population having local genetic makeup could result in a well-adapted population with similar morphological and ecological characters to local types.

3. Translocations in insects

The translocation of insects and other invertebrates has received considerably less attention than vertebrates; thus, not many examples of insects were translocated. However, ex situ conservation has become recognized as a more important technique for conservation for insects. With small body size, high reproductive rates, and short generation times, the insects have high potential to breed in mass captivity involving lower maintenance costs. Pearce-Kelly et al. suggested that the easy-breeding species with large captive populations have high potential for successful reintroduction programs [50]. The summary of 134 terrestrial insect translocations demonstrated that the proportion of success (52%) was higher than other animals while failed translocation programs were lower, 31% [51]. Thus, insects are the group most frequently considered in future translocations [52].

The objectives of insect translocation were classified into two groups, for conservation of the rare species and for socio-economic benefits of the flagship species.

Examples of the rare insect translocation are two vulnerable crickets, *Gryllus campestris* and *Decticus verrucivorus*, in England [53–54], the threatened tiger beetle *Cicindela dorsalis dorsalis* [55], a rare damselfly *Ischnura gemina* [56], Quino checkerspot butterfly *Euphydryas editha quino* [57] and the Genji firefly *Luciola cruciata* [58] (**Table 1**). With several iterations of releasing, the released insects could establish over a period of time and produced subsequent self-sustaining populations. The failure of translocation cases were caused by small released populations, disease infection, high dispersal stage used for releasing, low quality of habitat and weather conditions when releasing. The previous study [59] analyzed the documentations of 50 reintroduction activities of butterfly species and concluded that the successful projects had a higher number of attempts (per species) (11.1 ± 11.3 times for successful and 3.5 ± 3.2 times for unsuccessful programs). Successful programs introduced at least 292 individuals per reintroduction and continued for three years. Significantly, captive breeding was recommended for reintroduction programs for almost 50% of butterfly species.

As a dominant invertebrate flagship, the translocation of butterflies could be effectively used to build public awareness using live exhibits of butterfly farms. Many exotic butterflies were large-scale bred and imported across countries and regions for exhibition. If the butterflies come from similar environmental conditions and habitats, they might have high potential to establish in the new habitats. Consequently, the unintentional translocation might happen and cause ecological

Insects	Threats	Sources of translocated population	Success?	Problems of the translocation
Field cricket *G. campestris*	Rare and fragmented habitats	Captivity	Success (5 years)	- disease infection - cannibalism
Wart-biter bush cricket *D. verrucivorus*	Rare and fragmented habitats	Captivity	Failure	- high mortality rate in captivity result in small translocated population - high rearing cost
Tiger beetle *C. dorsalis dorsalis*	Sandy beach habitats of larvae were destructed from increasing of recreational activity.	Field collection (larvae)	Success (8 years)	- failure in adult translocation because of high dispersal behavior - larval predation by gulls
Damselfly *I. gemina*	Habitat structure changes and water area destruction from urbanization	Field collection (mating pairs)	Success (1 year in beginning phases)	- habitat changes from over vegetation in 2nd year. - unsuitable handling and marking techniques
Quino checkerspot butterfly *E. editha quino*	Habitat loss, fragmentation and extinction of native host plants	Captivity	Success	N/A
Genji firefly *L. cruciata*	Habitat loss, water pollution and tourism activities	Field collection and captivity	Success (70 years)	- harvested high amount of fireflies and released the non-native populations

Table 1.
Comparison of factors in some examples of rare insect translocation programs.

impact [60]. The opposite effect also may result, that captive bred populations lose the ability to live in natural habitats. After breeding in captivity for 100–150 generations, the large white butterfly have developed adaptive characters to captive conditions, i.e., heavier, higher ovary mass, higher numbers of laid eggs, and smaller wings that could decrease the butterflies' ability to re-establish in the wild [61].

The firefly is also a potential flagship to stimulate conservation awareness and action to support habitats for fireflies and other sympatric invertebrates. Apparently, firefly populations have declined or become extinct in many areas due to the impact of anthropogenic activities (i.e., habitat destruction, fragmentation, pollution and urbanization). Fireflies can be used to help promote public awareness and concern for biological diversity conservation.

The history of firefly translocation probably began in Japan [58]. The famous case happened in Tatsuno, Nagano prefecture where several thousand of the non-native Genji fireflies from Shiga prefecture were released as a tourist attraction. Subsequently the variation in flashing behavior and population genetics were investigated. Although the population of Genji fireflies in Tatsuno could self-establish over 70 years in the translocated area and bring more than 100,000 tourists a year, the native populations might be destroyed or lose genetic diversity. That is the risk under environmental change in the upcoming global crisis. Later, the scientists raised awareness of the firefly conservation issue and recommended the approach of using habitat preservation instead of artificial habitat creation for tourism. The fireflies were commonly labeled as an indicator species for environmental conservation. The translocation of captive fireflies in recovering polluted environments received more attention and resulted in appearance of 540 firefly events throughout Japan.

4. Genetic variation among firefly populations: the difficulty in translocation

Genetic issues become more important in sustainable biodiversity conservation especially in animal translocation. Avoiding or reducing genetic problems is a key to reducing the risk of extinction. Thus, not only focusing on maximizing species survival from established population measures, but also focusing on the genetic diversity, genetic drift and genetic adaptation to captivity are necessary to evaluate viability of populations in the long term.

The evidences of genetic and behavioral variation among firefly populations in Japan were discussed above. Firefly translocation requires an appropriate evaluation prior to their introduction into the wild. Likewise, the long term post-monitoring of both genetic and phenotypic measures is needed to measure the success of translocation and to identify future threats.

Genetic differentiation of fireflies is caused by various factors, including limitation of dispersal activity, habitat specificity or mating systems. The species with limited dispersal species have a higher probability of reproductive isolation. As in the desert firefly *Microphotus octarthrus*, which have winged males and apterous larviform females, the discontinuous habitats results in genetic isolation [62]. Strong habitat specificity was apparently involved, and there are several other cases of genetic divergence of fireflies influenced by geographical isolation. The variation of genetic structure of *Pyrocoelia rufa* in Korea was examined among islands, western and earthen parts being separated by mountain barriers resulting in different habitat types [63]. Consistently, the variation of genetic and phenotypic patterns of several firefly species in Japan was geographically separated by the Itoigawa-Shizuoka tectonic line. *Hotaria parvula* with morphological variation of body size are associated with genetic differentiation and are reproductively isolated [64]. Likewise, two population groups of *L. cruciata* in eastern and western areas of the tectonic line were also genetically different and displayed different flash communication patterns (slow-flash and fast-flash types) [65]. The variation in male flash patterns (based on inter-flash interval) was subsequently confirmed to have the potential to hinder in pre-mating between populations. The intermediate flash type fireflies that might be introgressive hybridization were found near the barrier area [66, 67]. Surprisingly, the "quick-flash type" was investigated in the Goto islands, the western tip of Kyushu but it was in the same haplotype as the fast flash fireflies inhabiting the mainland [68]. On the other hand, *A. lateralis* populations throughout the Korean Peninsula, northeast China, Sakhalin, and Japan were examined for genetic variation of two flash pattern types (which also have a difference in adult emergence season duration) but they could not be separated phylogenetically [69].

5. A case study of genetic and behavioral evaluation of Thai firefly species, *Sclerotia aquatilis*

5.1 Background

Sclerotia aquatilis (*L. aquatilis*) [70] is an aquatic firefly species. Individuals are commonly found in freshwater habitats throughout Thailand, i.e., ponds, ditches, wetlands inhabited by an abundance of aquatic snails and aquatic vegetation such as duck weed, water lettuce, water hyacinth, *Typha* spp., water lily, and Indian lotus. It is a multivotine species appearing all year round with the life cycle duration of 3–5 months [71], **Figure 1**. The larvae live in the water by respiring mainly through a pair of caudal spiracles to receive the air from water surface. They are frequently found back swimming at the surface of water.

The species has high potential for reintroduction programs because of the successful rearing technique developed [15, 16] and their several adaptive characteristics that support recovery of the new populations in old/new habitats. Since *S. aquatilis* occurs throughout Thailand, the reintroduction programs are probably

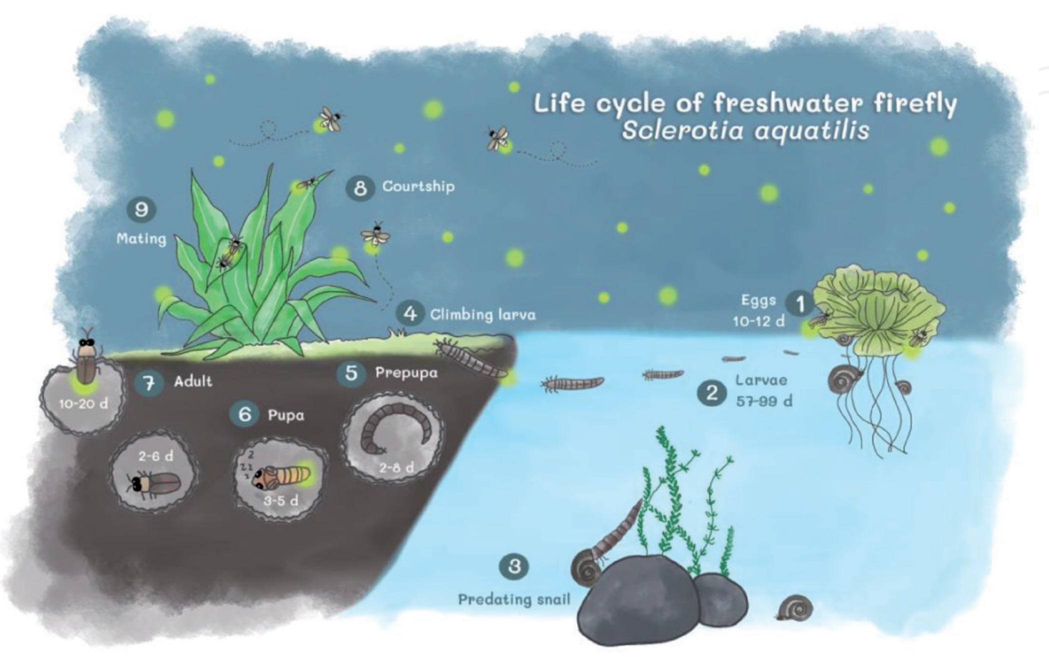

Figure 1.
Life cycle of S. aquatilis.

applied widely in the country. The firefly translocation has not previously been reported for this species.

There are many reasons suggesting genetic differentiation among *S. aquatilis* populations could lead to negative impact on translocation programs. Although geographic isolation frequently results in reproductive isolation by limiting gene flow between populations, it still remains unknown for firefly populations in Thailand. The expansion of cities and associated infrastructures not only destroy firefly habitats, but also creates habitat fragmentation. *S. aquatilis* populations are restricted to freshwater habitats, i.e., ponds, wetlands, and ditches. Adult female fireflies lack strong flight ability; therefore, habitat fragmentation seriously limits the range of their dispersal efforts, resulting in little immigration and even local extinctions. These limiting dispersal factors cause an increased the level of inbreeding and minimize interbreeding among spatially isolated populations. Thus, the probability of inbreeding and low genetic variability in nature is high in fragmented habitats. There is evidence of loss of genetic variation and the extinction of populations from habitat fragmentation in a butterfly metapopulation [72]. In addition, most *S. aquatilis* habitats overlap with human-used areas such as residential and agricultural areas, fireflies are subjected to many negative impacts from human urbanization, especially light pollution that can interfere with the sexual communication signals. Moreover, light pollution can be an effective dispersal barrier of fireflies. All these factors might result in both decreasing numbers and promoting inbreeding effects in populations.

5.2 Materials and methods

5.2.1 Study areas

During the process of urbanization, habitat loss and fragmentation have subsequently expanded particularly in Bangkok (BKK) area, where is the focus area for firefly reintroduction in this study. Historically, *S. aquatilis* inhabited in high abundance in the agricultural diches and ponds in the Chao Phraya delta area. However, the recent populations of the species have been decreased and become rare. The sources of translocated populations were from four nearby provinces, Samut Prakarn (SPK), Pathum Thani (PTE), Nakhon Pathom (NPT), and Suphan Buri (SPB) (**Figures 2** and **3**). Seven populations of fireflies from five locations were collected. One population from each province but two subpopulations from Pathum Thani (PTE2) and Nakhon Pathom (NPT2).

5.2.2 Firefly collection and maintenance

The collection of *S. aquatilis* specimens was conducted in all five locations during firefly season from August to November in 2012–2013, which was during the end of the raining season and the beginning of winter. The adult fireflies were collected at nighttime using a sweep net over freshwater areas. Adults were maintained in insect rearing cages supplied with a 10% honey solution on balls of moist cotton. In case of small populations, aquatic firefly larvae were also collected for molecular work. After observing the flashing behavior, the firefly specimens were placed in vials containing 100% ethanol, and stored in a − 80°C freezer prior the molecular study.

5.2.3 Genetic analysis

Genomic DNA from the hind legs of the adult specimens was extracted following the manufacturer's protocol using the DNeasy Blood & Tissue

Figure 2.
Map of Thailand the S. aquatilis study sites. The map illustration was modified from Vemaps.com.

Kit (Qiagen). A region encoding mitochondrial cytochrome c oxidase subunit II (COII) was amplified by the polymerase chain reaction (PCR) using the primers 5′-ATGGCAGATTAGTGCAATGG-3′ (TL2-J-3037) and 5′-GTTTAAGAGACCAGTACTTG-3′ (TK-N-3785) [69]. The PCR amplifications were performed as follows: an initial denaturing step at 94°C for 1 min, followed by 35 cycles beginning with a denaturation step at 94°C for 30 sec, an annealing step at 50°C for 30 sec, an extension step at 72°C for 1 minute, and a final step at 72°C for 10 min. The PCR product was verified by running through a 1% TAE agarose gel, stained with ethidium bromide and observed under UV light. The PCR product was treated with ExoSAP-IT PCR clean up reagent (Thermo Fisher Scientific, Massachusetts, USA) and sequenced by the 3130xl Genetic Analyzer (Thermo Fisher Scientific) with the BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific). The nucleotide sequences were assembled and edited individually using DNASIS Pro (Hitachi Software Engineering, Tokyo, Japan).

The numbers of base differences per site among sequences (p-distance) were calculated and constructed Unweighted Pair Group Method using arithmetic Average (UPGMA) tree using the p-distance by Molecular Evolutionary Genetics Analysis software (MEGA X) [73].

Median-joining networks among firefly haplotypes were constructed and post-processed under maximum parsimony in Network Version 4.6.1.1 (available at http://fluxus-engineering.com/sharenet.htm) to describe phylogeographic and genetic relationships between haplotypes.

5.2.4 Flashing behavior analysis

The live adult fireflies from each population were brought to the laboratory (26°C) for recording flash patterns within 1–2 days after collection to decrease the error from weakness and death. They were paired 1: 1 for mating in a mating arena that was prepared from a 7.1 × 11.0 × 6.5 cm of transparent plastic box with small moist cotton. They were allowed to have an adaptation period for 15–30 min before

Figure 3.
Habitat characteristics of the firefly collection sites, a) SPK, b) PTE, c) PTE2, d) NPT, e) NPT2 and f) SPB.

starting the experiment. The experiment was carried out under dark conditions (0 lux) for 30 min to 2 hr. after sunset.

The flashing communication was recorded using a Sony Handycam™ digital camera recorder (HDR-SR11E) at nightshot mode. All experimental mating boxes were separated from one another by placing black partitions between each arena to prevent flash interference from other mating pairs. Ten to 15 mating pairs from each population were randomly selected for video recording. Two flash types, courtship and warning flash types (**Figure 4**), which appeared at different periods of mating sequences, were recorded. The "courtship flashes" produced during courtship in responding to females, perhaps displayed during dorsal mounting. On the other hand, the brighter flashes displayed mostly during copulation called were defines as "warning flashes." At least 15 sec intervals or 30–50 flashes were recorded from each male. In case of small populations that had low numbers of females, the males were allowed to mate with virgin captive females to stimulate courtship behavior.

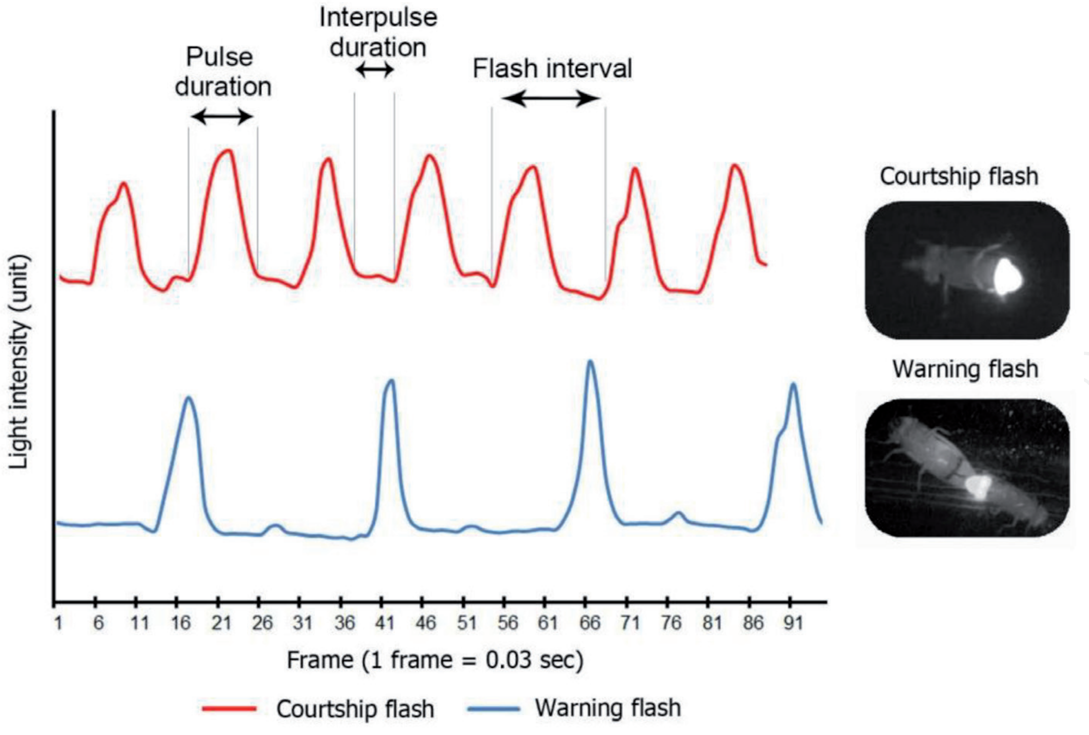

Figure 4.
Flashing behavior of male fireflies, courtship flash type (upper) and warning flash type (lower).

The video files were converted to audio video interleave or. AVI format files to analyze the flash parameters using time-lapse image analysis (TiLIA), a free software package for signal and flight pattern analyses of fireflies (available at Google Drive: https://drive.google.com/open?id=0B2o7FRVs2VohMmx2QzBVX3ZDeDA) [74] following the technique used by Thancharoen and Masoh [75]. The flash analysis was classified into three parameters, pulse duration, interpulse duration and flash interval, following previous study [76].

5.2.5 Statistical analysis

At least 30 flashes of courtship and warning flashes from each male were statistically analyzed. The pulse duration, interpulse duration, and flash interval among study sites were compared using One-way ANOVA and Tukey's multiple comparison tests. A value of $p < 0.05$ was considered statistically significant. The relationship between pulse and interpulse durations was tested using Pearson's correlation. All statistical analysis was performed using SPSS program version 24.

5.3 Results

5.3.1 Flashing behavior analysis

During mating behavior of *S. aquatilis*, the pulse durations of both courtship and warning flash types were quite similar, whereas the interpulse duration of warning flashes were twice longer than courtship flashes (**Table 2**). The correlation analysis of interpulse duration and pulse duration in each population showed that both flash parameters were negatively correlated (r in the range of −0.767 to −0.329, $P < 0.05$, n = 13). In case of short pulse duration, the interpulse duration was observed to be prolonged, stabilizing the flash interval.

The comparison of courtship flash parameters of all seven populations from five provinces showed that the fireflies from Suphan Buri province displayed different

Flash parameter	Duration in frame unit (mean ± SE)	
	Courtship flash (n = 60)	Warning flash (n = 28)
Pulse duration	5.54 ± 0.11	6.03 ± 0.17
Interpulse duration	6.78 ± 0.10	18.91 ± 0.34
Flash interval	12.32 ± 0.15	24.95 ± 0.38
Flash frequency	8.18 ± 0.09	4.03 ± 0.58

Table 2.
Flash parameters of courtship and warning flash types of S. aquatilis *(from overall populations).*

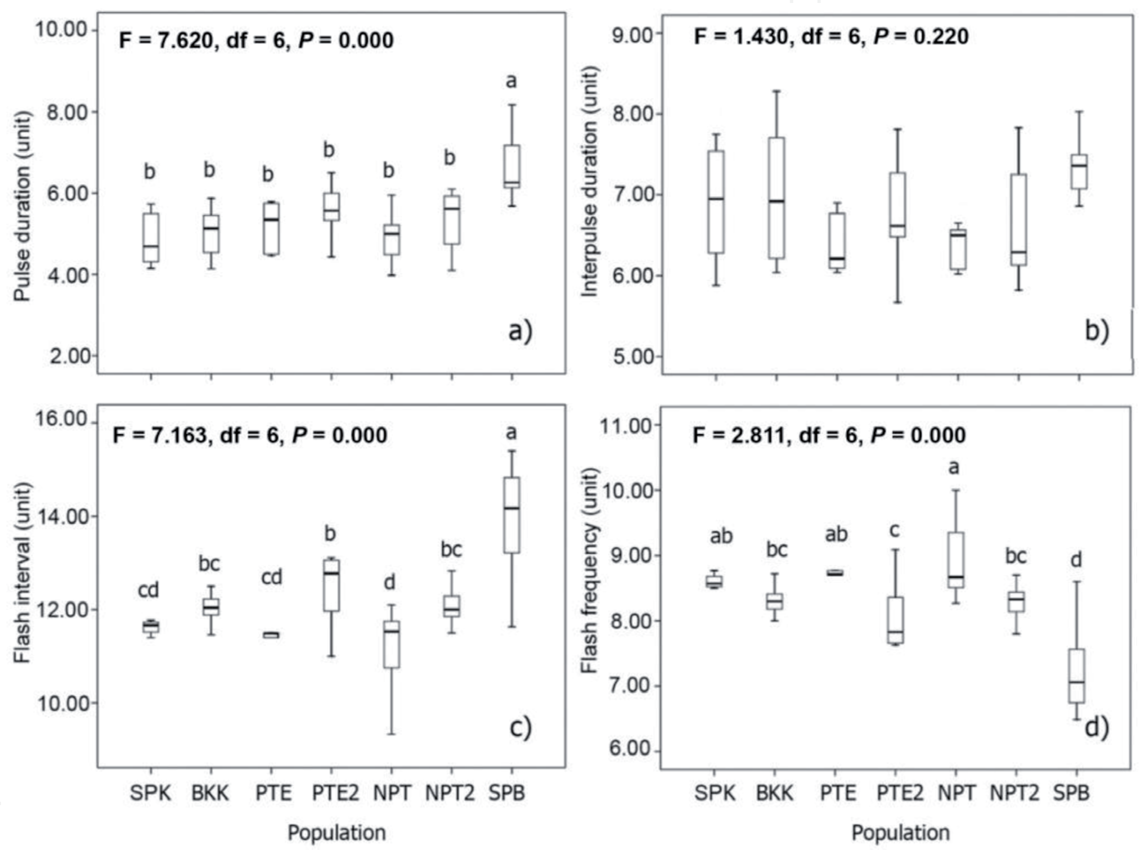

Figure 5.
The comparison of courtship flash parameters among seven populations of S. aquatilis; different letters indicate significant differences among different populations. Samut Prakarn (SPK), Bangkok (BKK), Pathum Thani (PTE), Nakhon Pathom (NPT), and Suphan Buri (SPB).

courtship flashes from the other sites located in the Bangkok Metropolitan Region (Samut Prakarn, Pathum Thani, Nakhon Pathom and Bangkok) (One-way ANOVA, $P < 0.05$; **Figure 5**). Results indicated that the Suphan Buri population had significantly longer pulse duration and flash interval resulting in slow flashing.

The flash parameters of the warning flash type could not be analyzed in all populations because not all experimental mating pairs displayed warning flashes. Therefore, only three populations from Pathum Thani, Nakhon Pathom and Suphan Buri province were analyzed. Perhaps because the mating happened under controlled environments without interference from mate competition and predation. Again, the Suphan Buri population flashed significantly differed when compared with other populations (**Figure 6**). It had a significantly long interpulse duration that resulted in having a long flash interval and a low flash frequency.

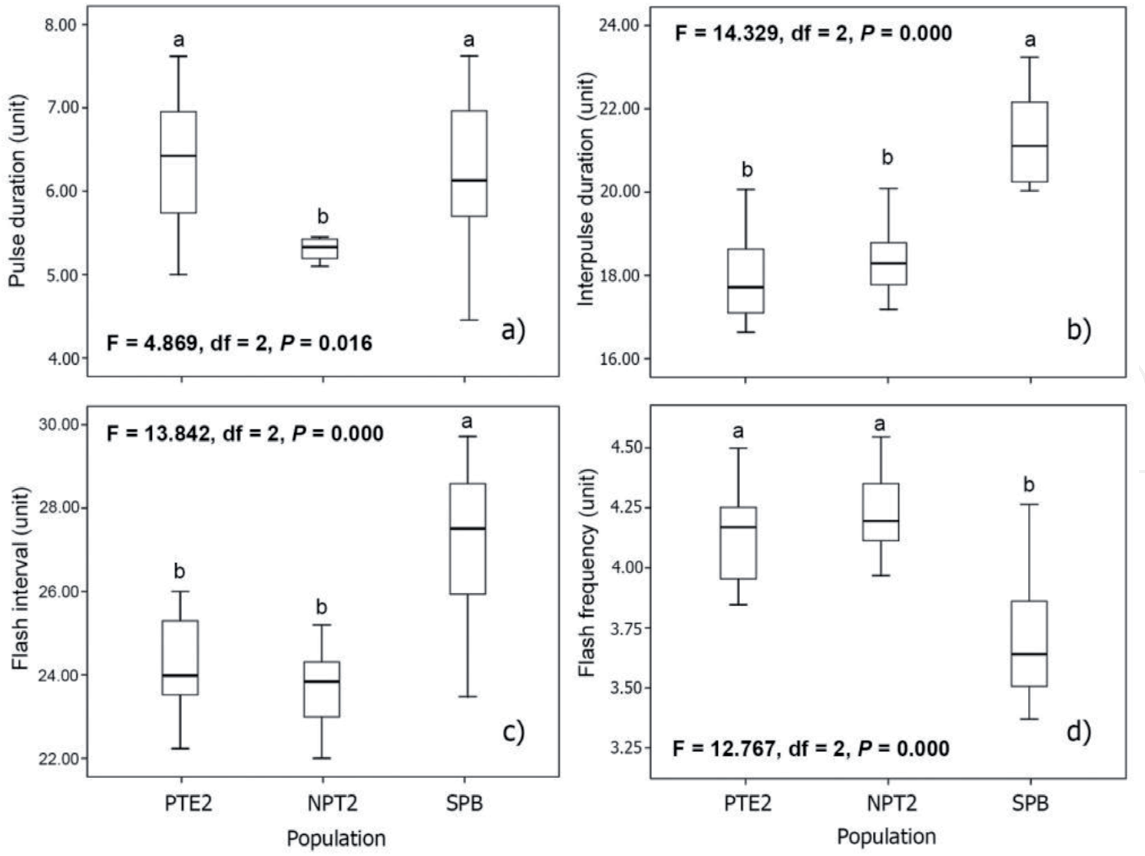

Figure 6.
The comparison of warning flash parameters among three populations of S. aquatilis; different letters indicate significant differences among different populations. Samut Prakarn (SPK), Bangkok (BKK), Pathum Thani (PTE), Nakhon Pathom (NPT), and Suphan Buri (SPB).

5.3.2 Genetic diversity of S. aquatilis

The genetic diversity of COII gene in *S. aquatilis* populations were examined from 132 individuals from seven locations in five provinces in the central part of Thailand. The sequences were registered in GenBank accession nos. MW800771 to MW800823 and MW814512 to MW814587. The p-distances among individuals ranged from 0 to 0.0122. The UPGMA tree revealed that regional cohesion of sequence types was not observed due to short p-distances (data not shown). The median-joining haplotype network was needed to confirm the low genetic diversity. The network revealed 37 haplotypes but not any phylogeographic sub-structuring of the firefly populations (**Figure 7**). Thus, no genetic differentiation was shown among the *S. aquatilis* populations examined.

5.4 Discussion

The study revealed flash signal variation among populations of *S. aquatilis* in the central part of Thailand. However, a distant population in Suphan Buri province apparently displayed longer pulse duration in the courtship flashes and longer interpulse in the warning flashes. As sexual communication, the pulse duration of the courtship signals is generally quite similar, preserving constant species-specific flash patterns. Most researchers studied "interflash interval" to define flash type from frequency, for instance, slow-flash, fast-flash, intermediate-flash and quick flash types [65–68]. However, the negative correlation between interpulse duration and pulse duration might help to balance the flash interval and flash frequency.

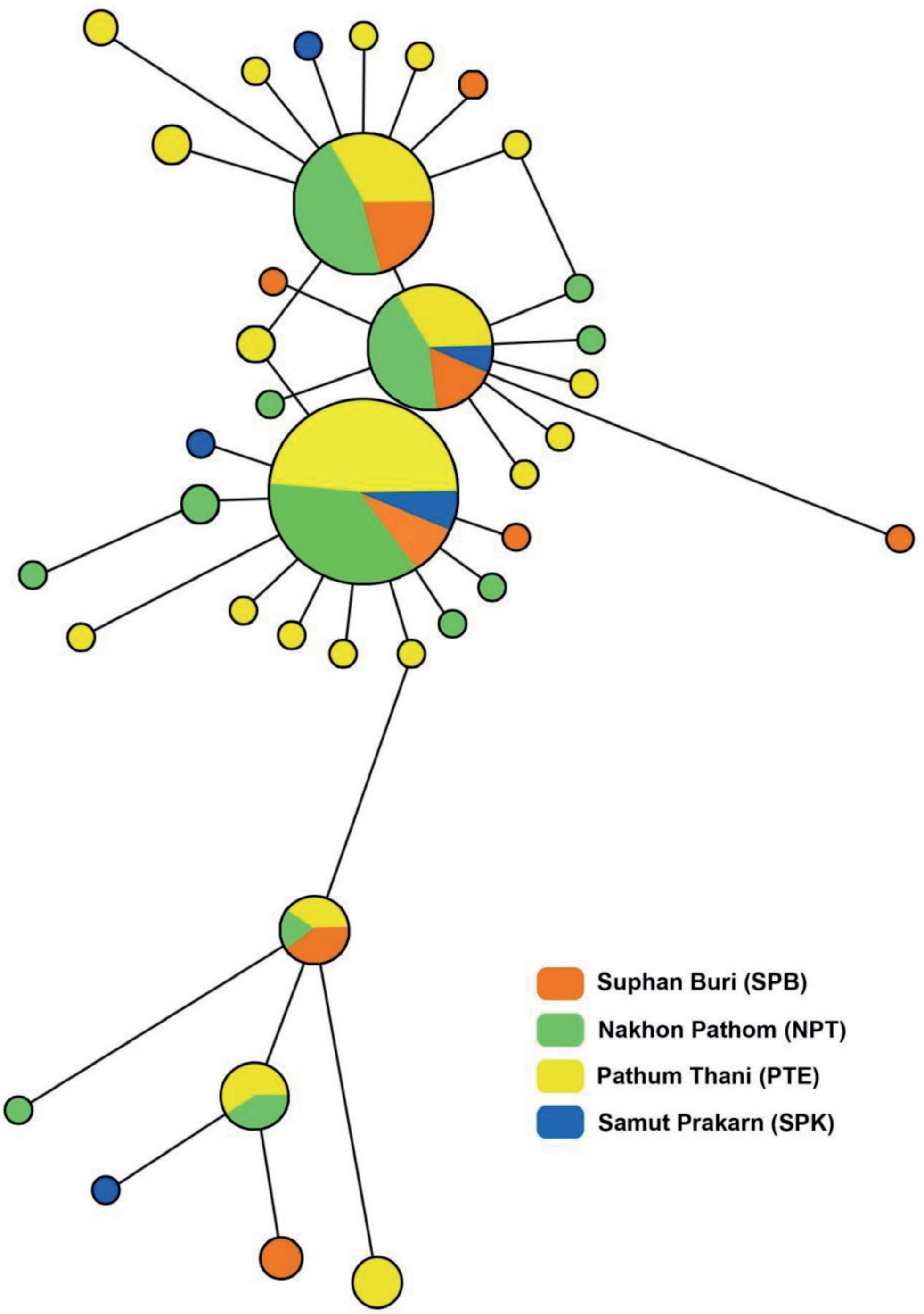

Figure 7.
Median-joining haplotype network generated from COII data from S. aquatilis collected from four locations in Central Thailand, different colors represent different collecting locations, sizes of nodes and pie segments are proportional to haplotype frequency, and length of branches is proportional to number of mutational changes between haplotypes.

Our finding was that there is intraspecific variation in flash communication of *S. aquatilis*. The fireflies in the Bangkok Metropolitan Region were fast-flash populations whereas the Suphan Buri population was slow-flashing although they did not show genetic differences among populations. This result is similar to the case of *L. lateralis* that *L. lateralis* populations distributed throughout the Korean Peninsula, Northeast China, Sakhalin, and Japan, the two flashing behavioral types could not be separated phylogenetically [69]. However, among populations with different flash types of *L. cruciata* in Japan, the genetic variation associated with flashing behavior was investigated [65, 67, 68]. The geographical differences caused by a great rupture zone of Japanese Islands might have had a strong effect on this species. Similarly, as the most geographically distant location of our studied populations, the Suphan Buri population (109 kilometers from Bangkok), is probably isolated from the others. Although there are no geographical barriers influencing allopatric populations like in the Japanese case, habitat fragmentation including light pollution barriers probably significantly affect the firefly populations. *S. aquatilis* fireflies normally inhabit in or near freshwater areas, the active males can fly fast and travel a long distance, the inactive females remain near a water area. The reduced female mobility behavior might limit the dispersal ability of the species and result in population isolation. In addition, artificial night lighting could also interfere with flashes of *S. aquatilis* resulting in adaptive behavior to adjust their flashes.

The fireflies inhabiting the area of the Bangkok Metropolitan Region might face a habitat flooded with artificial light that causes reduced ability to communicate with their mates. Selection pressure favors adaptations of their flash pattern to minimize light competition or to increase the clarity of flash signals to improve their mating success. It might be possible that the environmental selection pressure happened in the fireflies. The plasticity of the flashing behavior depending on situation and environmental conditions were examined in many firefly species [75, 77, 78]. The fireflies in light polluted areas will modify their flash patterns to be faster to mitigate steady light from artificial night lighting. Similar adaptations occur in acoustically communicating animals, where ambient noise, especially anthropogenic low-frequency noise, affected acoustic communication in blackbirds [79], tree frogs [80], tree swallows [81], fish [82] and tree crickets [83]. The birds sing louder with higher frequencies to mitigate low frequency traffic noise, while the males of the tree crickets shortened their calls (echemes) and paused singing with a higher probability with increasing noise level without modification of song frequency or interecheme interval. Unfortunately, no work has been done on their genetic differences between the normal and noise polluted populations.

5.5 Recommendations

Generally, genetic differentiation among populations would happen in a heterogenous or mosaic environment by reduction of population size, genetic drift, gene flow and natural selection and accumulated by geographic isolation. Although there is no geographical isolation in the central region of Thailand, in case of *S. aquatilis*, gene flow is limited by the dispersal ability of adult females and aquatic larvae that are restricted to the aquatic ecosystems. In addition, the light pollution is likely an important barrier limiting the adult dispersal whereas habitat fragmentation reduces population sizes, reduces habitat size of firefly larvae and increases isolation of small subpopulations. The wild populations of the fireflies are at risk of extinction due to the effect of inbreeding depression.

The recommendation for *S. aquatilis* translocation is to consider: (i) no genetic differentiation between the local and the released populations, (ii) no divergence in flash signals to prevent pre-mating isolation between recipient and donor populations, (iii) the distance between populations might promote variation among populations; thus, closer populations are properly used for translocation, (iv) the sources of translocated populations come from a large population or several subpopulations to acquire proper numbers of source populations and decrease the effect of inbreeding depression. In addition, other factors, for example, habitat quality, source of translocated fireflies (from wild or captivity), released stage, frequency of releasing, released area and other environmental conditions during releasing, can relate to the success of program. This information is probably species specific; therefore, the biological and ecological characteristics of the focus species are needed for translocation application. Significantly, the long-term monitoring of establish populations also is necessary.

In the case study, although the *S. aquatilis* populations in the central part of Thailand have no genetic divergence among populations, the variation of flash signals was found in a location of Suphan Buri province. The translocation of the species could happen if the donor and recipient populations come from Bangkok Metropolitan Region where the fireflies displayed similar flash signals and no genetic divergence among populations.

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Conflict of interest

The author declares no conflict of interest.

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