Comparative Population Genetics of Two Korean Aedes Mosquito Species with Vector Potential based on Mitochondrial DNA

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Research

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Abstract

**Background:** Mosquitoes of the genus *Aedes* are important invasive species contributing to the spread of chikungunya, dengue fever, yellow fever, Zika virus, and other dangerous vector-borne diseases. *Aedes albopictus* is native to southeast Asia with rapid expansion due to human activity, showing a wide distribution in the Korean peninsula. *Aedes flavopictus* is considered to be native to East Asia with a broad distribution in the region, including in the Korean peninsula. Gaining a better understanding of the genetic diversity of these species is critical for establishing strategies for disease prevention and vector control.

**Methods:** We obtained DNA from 148 specimens of *Ae. albopictus* and 166 specimens of *Ae. flavopictus* in Korea, and amplified two mitochondrial genes (*COI* and *ND5*) to compare the genetic diversity and structure of the two species.

**Results:** We obtained a 658-bp sequence of *COI* and a 423-bp sequence of *ND5* from the two mosquito species. We found low diversity and an insignificant population genetic structure in *Ae. albopictus*, and high diversity and an insignificant structure in *Ae. flavopictus* for these two mitochondrial genes. *Ae. albopictus* had less haplotypes with respect to the number of individuals, and a slight mismatch distribution was confirmed. By contrast, *Ae. flavopictus* had a large number of haplotypes compared with the number of individuals, and a large unimodal-type mismatch distribution was confirmed. Although the genetic structure of both species was insignificant, *Ae. flavopictus* exhibited higher genetic diversity than *Ae. albopictus*.

**Conclusions:** *Ae. albopictus* appears to be an introduced species, whereas *Ae. flavopictus* is an endemic species to the Korean peninsula, and the difference in genetic diversity between the two species is related to their adaptability and introduction history. As an endemic species, *Ae. flavopictus* is likely to have a larger population size than expected. Further studies on the genetic structure and diversity of these two mosquito species will provide useful data for vector control.

**Background**

Arthropod-borne viruses are transmitted by blood-sucking insects to animals and humans. Most of them, are transmitted by mosquitoes [1, 2]. There are 43 genera and 3,530 species of mosquitoes in the world, however, species belonging to the genera *Aedes*, *Anopheles*, and *Culex*, are the main vectors of mosquito borne diseases [3, 4]. In particular, mosquitoes belonging to the genus *Aedes* are becoming the main vectors for spreading fatal diseases such as chikungunya, dengue fever, yellow fever, and Zika virus, that often occur in Asian countries [4, 5, 6]. As mosquito borne diseases may grow in the future due to fast globalization and climate change, more information is needed [2, 7].

Mitochondrial genes are widely used in research on molecular evolution and population genetics of vector insects. Because they have a relatively high mutation rate, and high levels of polymorphism and divergence due to their inherent sensitivity, they are highly useful as molecular markers [8, 9, 10, 11]. Many vector studies have investigated where the population was introduced using mitochondrial genes [12, 13]. Population structure and genetic diversity between populations can affect vector capacity [14]. The understanding of these factors is necessary for vector control [15].
Aedes albopictus, originally from Southeast Asia, has recently spread all over the world except for Antarctica, and is considered one of the most dangerous alien species [16, 17, 18]. The first record of Ae. albopictus in South Korea was in 1940, and its distribution has recently expanded throughout the Korean peninsula [19, 20]. Together with *Aedes aegypti*, substantial research attention has been paid to *Ae. albopictus* as major players in vector-borne diseases [21, 22, 23]. The main reason for the global expansion is that larvae are introduced through used tires, bamboo, etc., due to human activities [24, 25]. Additionally, the range of habitats they can live in has widened as a result of the temperature rise due to global warming [26, 27]. *Ae. Albopictus*’ eggs have been shown to tolerate cold weather, and have the potential to expand its distribution in colder regions [28, 29].

*Aedes flavopictus* is original from East Asia, including Japan and South Korea, and is widely distributed in that region, but there are morphological and genetic differences depending on the geographical range [30, 31]. It has been found that *Ae. flavopictus*’ eggs can survive in colder environments than *Ae. albopictus* [32] and recently expanded its distribution from East Asia to European countries [33, 34, 35]. According to the results of continuous monitoring on the Korean Peninsula, the frequency of appearance of *Ae. flavopictus* is not high [20, 36, 37, 38]. *Ae. flavopictus* is not known to act as a vector like *Ae. albopictus* and other *Aedes* species, but it has previously been shown that it may propagate dengue fever [30, 39, 40].

Since the two species are distributed over a wide area in Korea and Japan and share a common habitat [31, 41, 42], attention over their overlapping distribution is gradually increasing, and it is said that there is a possibility of interspecific crossing [43, 44, 45]. Not only do the distributions overlap, but the two morphologies are similar [31, 35, 46], and Japanese studies have shown that the two are phylogenetically close to each other [47, 48]. As *Ae. albopictus* and *Ae. flavopictus* are closely related species and have similar ecological roles and habitats, they can be compared to each other.

The Korean Peninsula has various climates and geographical environments, and the diversity of arthropods that transmit arthropod-borne viruses is also high [49, 50]. There are 11 genera and 56 species of mosquitoes in Korea, including 19 species in the genus *Aedes*. The presence of *Ae. albopictus* and *Ae. flavopictus* was recorded in Korea in the past [19, 51, 52]. Since malaria and Japanese encephalitis occur frequently in Korea, only studies have focused on the vectors of these conditions, and the genus *Aedes* has not been investigated [53, 54, 55]. There are cases in which foreign mosquitoes have become indigenous bringing infections from abroad. Additionally, but Korea also has steadily imported patients, so it is not possible to say that it is a clean country for viruses mediated by *Aedes*, so it is necessary to establish a preemptive control strategy [56, 57].

This study compared the genetic diversity and structure of two species of *Aedes* mosquitoes living in Korea through two mitochondrial genes with the aim of monitoring mosquito populations. With this work, we intend to create basic data to establish vector control strategies.

**Methods**

**Sampling and DNA extraction**

A total of 314 individual mosquitoes were sampled in Korea between 2017 and 2020, including 148 individuals of *Ae. albopictus* from 19 locations and 166 individuals of *Ae. flavopictus* from 14 locations. Adult mosquitoes were collected using nets and BG-Sentinel traps (Biogents AG, Germany). Specimens were individually preserved in
tubes filled with 80% ethanol and stored at 4°C until DNA extraction. DNA was extracted from one to three legs of each sample using DNeasy Blood & Tissue (Qiagen, Valencia, CA, USA).

Polymerase Chain Reaction (PCR) And Sequencing

Two regions of mitochondrial genes (COI and ND5) were amplified by PCR using the following primer pairs: albCOIF (5'-TTTCAACAAATCATAAGATATTGG-3') and albCOIR (5'-TAATCTTGAGATGACCAAAAATCA-3') for COI [58], and ND5_6500F (5'-TCTTATAAGATAATCCCACG-3') and ND5_7398R (5'-TCTTCTGCTTTAGTTACTATTTCC-3') for ND5 [59]. For COI, PCR amplifications were performed in a 25 µL reaction volume containing 0.5 µL of isolated DNA, 2.5 µL of 10× Taq buffer, 2.0 µL of MgCl2 (25 mM), 0.7 µL of dNTP solution (2.5 mM each), 0.5 µL of each primer, and 0.3 µL of Taq DNA polymerase (Takara Bio Inc., Kusatsu, Shiga, Japan). The PCR cycling conditions were as follows: an initial denaturation step at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 45°C for 30 s, and elongation at 72°C for 45 s; with a final extension at 72°C for 7 min. For ND5, the PCR mixture was the same as that used for COI. The amplification conditions were as follows: initial denaturation at 98°C for 5 min; followed by 10 cycles at 95°C for 1 min, 45°C for 1 min, and 72°C for 1 min 30 s; 30 cycles at 95°C for 1 min, 46°C for 1 min, and 72°C for 1 min 30 s; and a final extension at 72°C for 3 min. PCR products were separated by 2% agarose gel electrophoresis (Sigma Aldrich, Germany) and sequenced by Cosmo Genetech (Seoul, Korea) using ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Data Analyses

The sequences of the two mitochondrial genes were aligned using the ClustalW plugin on Geneious Prime 2020.1.2 (https://www.geneious.com) and prepared as concatenated sequences. DnaSP 6.12.03 [60] was used for the genetic diversity analysis of mitochondrial DNA, in which the number of haplotypes (H), number of segregating sites (S), haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (k) were examined.

Pairwise F_{ST} values were estimated using Arlequin 3.5 software [61] to investigate genetic differentiation among the populations. Principal coordinates analysis (PCoA) was performed with GenAlEx version 6.51b2 [62] based on pairwise F_{ST} values.

Analyses of molecular variance (AMOVA) were performed using ARLEQUIN 3.5 [61] with the locus-by-locus option and using 1,000 permutations to identify the population structure. Specimens were grouped according to regional groups in South Korea: Group 1 comprised specimens from Gyeonggi-do, Group 2 comprised specimens from Gangwon-do, Group 3 comprised specimens from Chungcheong-do, Group 4 comprised specimens from Gyeongsang-do, and Group 5 comprised specimens from Jeolla-do.

To better understand the genealogical relationships, the haplotypes were constructed using the TCS method as implemented in PopART 1.7 [63].

To investigate the demographic history of populations, deviations from selective neutrality were tested by Tajima's D [64] and Fu's FS [65] metrics using Arlequin 3.5 [61]. To confirm whether a population had undergone sudden expansion, a mismatch distribution was determined using DnaSP 6.12.03 [60].
Results

Mitochondrial gene sequence analysis resulted in a CO1 sequence of 658 bp and an ND5 sequence of 423 bp in the 19 populations (148 individuals) of Ae. albopictus, and sequences of the same length were obtained for the 14 populations (166 individuals) of Ae. flavopictus (Supplementary Table 1). In the two mitochondrial DNA concatenated sequences, there were 25 haplotypes in Ae. albopictus and 107 haplotypes in Ae. flavopictus.

The genetic diversity analysis revealed a relatively low number of haplotypes in Ae. albopictus compared to the total number of individuals, with relatively low haplotype diversity (0.396) and nucleotide diversity (0.00075) in the entire population. The highest haplotype diversity was found for the 2018 Anyang and Gyeongju populations, and the lowest values were 0 for six populations. The 2018 Anyang and Gyeongju populations also exhibited the highest nucleotide diversity. Ae. flavopictus showed a relatively high number of haplotypes compared to the total number of individuals, and high levels of haplotype diversity (0.990) and nucleotide diversity (0.00894) were found in the entire population. Analysis of the two species revealed that Ae. flavopictus exhibited higher levels in various genetic diversity indices.

Ae. albopictus showed insignificant pairwise F$_{ST}$ values overall, but the Geoje population showed a high level of significance in structure compared to the other populations. By contrast, Ae. flavopictus showed low overall F$_{ST}$ values, among which those of the Yeosu and Yeoncheon populations were significant (Fig. 2; Supplementary Table 2). In both species, there were no significant pairwise F$_{ST}$ values among domestic populations except for a few populations.

AMOVA showed low genetic variance among both species, but high variance within populations. In particular, Ae. flavopictus showed higher variance than Ae. albopictus, indicating that it can form a genetic structure within populations.

In the haplotype network, Ae. albopictus showed a simple star-like form, in which several haplotypes diverged from one of the largest haplotypes, and hap_1 had the highest frequency of 78% in all populations. Private haplotypes, most of which were singleton haplotypes, accounted for 22%. Ae. flavopictus exhibited a complex haplotype network, which was found to have a higher haplotype compared to the total number of individuals. Most of the network was composed of singleton haplotypes. When comparing the two species, Ae. flavopictus had more haplotypes than Ae. albopictus and showed a complex haplotype network.

With respect to demographic history, Ae. albopictus showed negative but low values for Tajima’s D (−0.36713) and Fu’s F$_S$ (−0.44574) in the whole population. Negative values of Tajima’s D (−0.34726) and Fu’s F$_S$ (−1.37746) were also found for the entire population of Ae. flavopictus. In both species, Ae. flavopictus showed slightly higher Fu’s F$_S$ values. For the mismatch distribution, the result of Ae. albopictus was insignificant, whereas Ae. flavopictus showed a large unimodal shape, indicating the possibility of sudden expansion of the population.

Discussion

Based on the above results, two conclusions can be drawn. Higher genetic diversity was observed in Ae. flavopictus than in Ae. albopictus, and the two species of mosquitoes generally showed low levels of genetic structure except for some populations.
There are two hypotheses addressing the overall low diversity of *Ae. albopictus* in Korea. The first is a hypothesis regarding the spread of *Wolbachia* virus to the mitochondria: Previous studies have shown that intracellular *Wolbachia* was detected in 17 populations in Korea, and these group are known to have low mitochondrial diversity [66, 67]. However, there is no clear evidence of *Wolbachia* in many samples, and further analysis of the nuclear genes, as well as the mitochondrial genes, will be needed to reveal the impact of *Wolbachia* on genetic diversity in the population [66, 68]. The second hypothesis is that the introduced *Ae. albopictus* may have been affected by Korea's harsh climate, which is different from that of the country of origin: *Ae. albopictus* is considered to be an invasive species that has recently spread abroad from its origin in Southeast Asia [16, 17]. These mosquitos have adapted to the environment of each country since their introduction, but previous studies have shown that they also have low diversity in the countries from which they were introduced [12, 69]. The environment in Southeast Asia, the native habitat of *Ae. albopictus*, is humid and high in temperature, facilitating the spread of *Ae. albopictus* [70]. However, winters are quite severe in Korea, with cold, dry weather. The average temperature is less than 10°C. This environment could lead to a decrease in the population size of this mosquito, leading to a decrease in genetic diversity [55, 71]. In the domestic populations, the Geoje population is different from other populations. This is believed to arise from genetic differences due to the physical distance between mosquitos introduced by human activities. The distance traveled by mosquitos in the natural environment is, however, only a few kilometers [72], and there are study results showing that the populations have been genetically structured in heterogeneous habitats due to their limited dispersive abilities [73].

Korean *Ae. flavopictus* have a high genetic diversity, and a complex haplotype network. There are two hypotheses regarding their high diversity. The first hypothesis is based on the fact that *Ae. flavopictus* is endemic to East Asia: *Ae. flavopictus* is known to be native to East Asia, and is divided into three subspecies depending on the region. The subspecies are morphologically and genetically distinct [30, 31]; *Ae. flavopictus, Ae. flavopictus downsi*, and *Ae. flavopictus miyari*. Among them, *Ae. flavopictus* is distributed in the Korean Peninsula, and records show that they have existed here for a long time, but there have been few molecular studies into this species, so the extent of its genetic diversity is not fully known [30, 51, 74]. Studies on mosquitoes of the genus *Aedes* show that genetic diversity in the original population is much higher, which supports the contention that the original population of *Ae. flavopictus* is here [75, 76]. The second hypothesis takes into account the adaptation to cold climates as an endemic species: *Ae. flavopictus* is an Asian species that does not exist in tropical regions, and lives in subtropical areas throughout the cool-temperate region [48]. This mosquito has recently been found in the Netherlands, a more northerly region, and is considered highly likely to spread due to its ability to cope with environmental changes [34, 35]. It has excellent environmental adaptability as well as an ability of the eggs to withstand cold conditions, and can exist in dry conditions for a long time [51, 77]. Studies have also shown that it is genetically close to *Aedes galloisi*, a northern mosquito species in the same genus [47].

The genetic structure of domestic *Ae. albopictus* and *Ae. flavopictus* has been found not only in these two species, but also in other mosquito species, and this pattern is common in mosquitoes [11, 78]. Differences in diversity between the two species can be explained in several ways. *Ae. albopictus* is an introduced species that shows high genetic diversity in its native Southeast Asia region. However, in Korea, these mosquitoes have a considerably lower genetic diversity. A small mismatch in distribution and one haplotype shared by various populations show that patterns observed in *Ae. albopictus* may have been affected by the decrease in effective population size, human introduction, and natural environment changes [12, 69, 79]. In future studies, vector control can be aided by the comparison of local mosquitoes with overseas populations, using various markers, which will facilitate the inference of the timing of introduction in Korea. *Ae. flavopictus*, an endemic species, has
high genetic diversity, and has a large unimodal mismatch distribution and a complex haplotype network. The unimodal form of its mismatch distribution indicates that the *Ae. flavopictus* population may have recently experienced a large population expansion. The successful distribution and increasing population of endemic species of *Ae. flavopictus* may have been affected by human demographics [22], and the complex form of the haplotype network indicates a high mutation rate, which can increase the rate of development of resistance in insects [80, 81]. The difference in genetic diversity between these two mosquito species living in Korea may also come from the differences in effective population size, due to their ability to adapt to the cold, as well as their status as an endemic or introduced species. Although the distribution of the two species overlaps, *Ae. albopictus* can survive for up to 24 hours at -10°C in the form of diapause eggs [82], and the eggs of *Ae. flavopictus* can survive for a longer period [32, 48, 51]. The decreased survival rate of eggs can affect the effective population size, since fewer adults develop [83, 84]. This difference in cold adaptation can affect the size of the effective population, which can lead to differences in genetic diversity [55, 85, 86]. Monitoring of vectors in Korea has shown that the frequency of *Ae. flavopictus* was not high, but the reason for the large potential population size in this study lies in the difference between the location and the method of collection [20, 36, 37, 38]. Continuous monitoring is needed, because this species is highly likely to affect humans, as it has a large population size and considerable potential as a vector.

The differences in the genetic diversity of *Ae. albopictus* and *Ae. flavopictus* populations revealed in this study suggest that continuous monitoring of these species with multiple possibilities as vectors is essential. To understand the genetic diversity of *Aedes* mosquito species in Korea, sampling in more diverse regions and the use of different genetic markers will be conducted in further studies.

**Conclusions**

This is the first paper comparing genetic diversity and the genetic structure of two *Aedes* mosquito species inhabiting Korea. This study found that *Ae. albopictus*, which is considered to be an introduced species, has lower genetic diversity than *Ae. flavopictus*, the endemic species. The low diversity of *Ae. albopictus* suggests that these mosquitoes were introduced by humans, but did not fully adapt to the environment of the Korean Peninsula. The high diversity of *Ae. flavopictus* could be due to its greater adaptability to the environment of the Korean Peninsula as an endemic species, but may also be influenced by an increase in population and resistance to pesticides. However, the Korean Peninsula cannot be free from mosquito-borne diseases due to the rise in temperature caused by climate change, domestic inflow of patients, and population density. This fundamental study of potential vector-like intermediates will be a useful foundation for vector control.

**Abbreviations**

COI
Cytochrome c oxidase subunit I
ND5
NADH dehydrogenase subunit 5

**Declarations**

*Availability of data and materials*
Accession numbers for mitochondrial DNA sequences generated in this study are in Supplementary Table 1.

Authors’ contributions

Jiyeong performed practical work and Jongwoo monitored the study. Jiyeong analyzed the data and drafted with Jongwoo. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Consent for publication

Not applicable.

Competing interests

Not applicable.

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Tables
| Species          | population | Sample size | Sample | H  | S  | k  | Hd   | π    | Tajima's D | Fu's $F_S$ |
|------------------|------------|-------------|--------|----|----|----|------|------|------------|------------|
| Ae.albopictus    | 2017_Wonju | 18          | 4      | 0.725 | 0.549 | 0.00067 | -1.12822 | -2.0958 |
|                  | 2020_Wonju | 18          | 3      | 0.333 | 0.111 | 0.00031 | -1.71304 | 0.65061 |
|                  | Yeoncheon  | 5           | 0      | 0    | 1   | 0   | 0    | 0    | 0          | 0          |
|                  | Yangsan    | 12          | 4      | 1.152 | 0.758 | 0.00107 | -0.45947 | -2.89747 |
|                  | 2020_Anyang| 4           | 1      | 0.500 | 0.500 | 0.00046 | -0.61237 | 0.17185 |
|                  | 2018_Anyang| 4           | 6      | 3.167 | 1.000 | 0.00293 | -0.31446 | -1.15708 |
|                  | Chuncheon  | 7           | 1      | 0    | 0   | 0   | 0    | 0    | 0          | 0          |
|                  | Cheongnyang| 2           | 1      | 0    | 0   | 0   | 0    | 0    | 0          | 0          |
|                  | Daejeon    | 8           | 1      | 0    | 0   | 0   | 0    | 0    | 0          | 0          |
|                  | Gwacheon   | 7           | 2      | 0.571 | 0.524 | 0.00053 | -1.23716 | -0.9218 |
|                  | Geoje      | 5           | 2      | 1    | 0.600 | 0.600 | 0.00056 | 1.22474 | 0.62615 |
|                  | Gwangju    | 2           | 1      | 0    | 0   | 0   | 0    | 0    | 0          | 0          |
|                  | Gyeongju   | 2           | 3      | 3.000 | 1.000 | 0.00278 | 0      | 1.09861 |
|                  | Jeung-do   | 5           | 2      | 1.000 | 0.800 | 0.00093 | 0.24314 | -0.47542 |
|                  | Jeonju     | 28          | 2      | 0.143 | 0.140 | 0.00013 | -1.5106 | -2.26798 |
|                  | Sokcho     | 13          | 2      | 0.308 | 0.295 | 0.00028 | -1.46801 | -1.40150 |
|                  | Seoul      | 3           | 2      | 1.667 | 0.667 | 0.00062 | 0      | 0.20067 |
|                  | Yeoju      | 3           | 1      | 0    | 0   | 0   | 0    | 0    | 0          | 0          |
|                  | Yeosu      | 2           | 1      | 0    | 0   | 0   | 0    | 0    | 0          | 0          |
| Ae.flavopictus   | 2017_Wonju | 6           | 19     | 8.667 | 1    | 0.00802 | 0.25884 | -1.18145 |
|                  | 2017_Uiwang| 21          | 50     | 15.281 | 0.971 | 0.01414 | 0.39649 | -1.61069 |
|                  | 2020_Uiwang| 10          | 29     | 9.089 | 0.978 | 0.00841 | -0.54358 | -1.86104 |
|                  | Yeoncheon  | 2           | 1      | 0    | 1   | 0   | 0    | 0    | 0          | 0          |
|                  | Yangsan    | 17          | 26     | 6.75  | 0.971 | 0.00624 | -0.49372 | -5.0955 |
|                  | Asan       | 22          | 49     | 9.779 | 0.939 | 0.00905 | -1.07423 | -2.0798 |
|                  | Bonghwa    | 18          | 25     | 5.693 | 0.987 | 0.00527 | -0.86027 | -9.17073 |
|                  | Chuncheon  | 2           | 9      | 9    | 1    | 0.00833 | 0      | 2.19722 |

Bold cases represent significant at $P < 0.05$
### Table 2
Analysis of molecular variance (AMOVA) of this study

| Species     | source of variation                  | d.f. | percentage of variation (%) |
|-------------|--------------------------------------|------|-----------------------------|
| Ae. albopictus | Among groups                         | 4    | -5.6                        |
|             | Among populations within groups      | 14   | 46.41                      |
|             | Within populations                   | 129  | 59.19                      |
| Ae. flavopictus | Among groups                         | 4    | 4.03                        |
|             | Among populations within groups      | 9    | 9.63                        |
|             | Within populations                   | 152  | 86.34                      |

Bold cases represent significant at $P < 0.05$