An Analysis of the Population Genetic Structure of *Cryptotympana atrata* (Hemiptera: Cicadidae) in South Korea Using Single-Nucleotide Polymorphisms from Direct Amplicon Sequencing[1]

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Abstract *Cryptotympana atrata* F. (Hemiptera: Cicadidae) is designated as a climate-sensitive indicator insect in South Korea. Notably, *C. atrata* populations are experiencing significant range expansion in response to rising temperatures, mainly from southern temperate regions, including a southern subtropical remote island, Jeju, to northern cooler areas. Therefore, this study aimed to explore the genetic diversity and structure of *C. atrata* populations across South Korea using 118 single-nucleotide polymorphisms obtained from direct amplicon sequencing. The most significant genetic differentiation was noted at ∼100-km distance of the remote Jeju island population from all the inland populations. This signifies the importance of long-standing physical isolation from biogeographic history, rather than ongoing global warming. Within the mainland, only a slight genetic distinction was detected in two of the three newly expanded populations in the northern cooler region, with similar genetic diversity in all inland populations, suggesting that an adaptation to northern lower temperatures was not the major factor facilitating the expansion to new areas. Instead, ongoing global warming seems effective enough to promote the expansion of *C. atrata* to the northern cooler regions through the facilitated dispersal, exploration of suitable habitats, and success, even under the unfavorable lower temperature conditions in the newly expanded area.

Key Words climate-sensitive indicator insects, global warming, single-nucleotide polymorphism, amplicon sequencing

Global warming profoundly influences ecologic and evolutionary processes in biodiversity worldwide (Thomas et al. 2004, Vittoz et al. 2013), causing organisms to respond differently, such as through phenological plasticity (Canale and Henry 2010, Zografou et al. 2021), shifts in latitudinal and elevational ranges (Forister et al. 2010, Jung et al. 2014, McCain and Garfinkel 2021, Vittoz et al. 2013, Wilson et al. 2007), range retraction and expansion (Maggini et al. 2011, Parmesan 2006, Thomas et al. 2006, Vandewoestijne and Van Dyck 2010), local increases (Grabherr et al. 1994, Klanderud and Birks 2003, Walther et al. 2005), and genetic adaptations

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(Barghi et al. 2019, Günter et al. 2019, Oostra et al. 2018). Nonetheless, predictions on future biodiversity appear discouraging, with one in six species, regardless of taxonomic group, anticipated to be at risk of potential extinction under the representative concentration pathway 8.5 scenario (Urban 2015). To alleviate this possibility, increased observations on the response of species in the distributional range and changes in population size could be pivotal for improved management of future biodiversity. Moreover, examining population genetic aspects could be important because the genetic diversity within species is the fundamental source of future biodiversity and adaptive assets against ecologic uncertainty (Frankham 1995, Hoffmann and Sgrò 2011).

Thus, in response to ongoing climate change, the National Institute of Biological Resources in South Korea (NIBR; 2017) designated 15 insect species as climate-sensitive biological indicators, among which the annual cicada, *Cryptotympana atrata* F. (Hemiptera: Cicadidae), is the only representative of the order Hemiptera. As ectothermic organisms with life histories tightly linked to temperature, insects are highly responsive to even subtle shifts in environmental conditions (Deutsch et al. 2008, Forrest 2016). Further, *C. atrata*, in particular, exhibits ecologic characteristics that heighten its sensitivity to climatic variation, such as a strong preference for higher temperatures during its multiyear nymphal stage, a reliance on elevated nighttime temperatures for calling behavior, short dispersal distance, and high fidelity to emergence sites (Karban 1981, Ki et al. 2016, Simões and Quartau 2007, Xie et al. 2023). Moreover, given the logistic and methodological challenges in comprehensive biodiversity monitoring, focusing on sentinel species with high ecologic sensitivity and clear range dynamics, such as *C. atrata*, could represent a practical and effective strategy. These features make *C. atrata* a valuable proxy for assessing the ecologic effects of climate warming on insect populations.

Cryptotympana atrata is found across the northern regions of Southeast Asia, including South Korea, and predominantly inhabits high temperate and tropical climates (Fukuda et al. 2006). *Cryptotympana atrata* was abundant in the southern regions of South Korea, including a southern remote island, Jeju, which is ∼100 km south of the Korean Peninsula; however, *C. atrata* was absent in the northern region, such as in Gyeonggi Province and Gangwon Province (Kim and Song 2017, Sun et al. 2018). Meanwhile, the *C. atrata* population increased gradually in mainland South Korea, and changes from global warming caused *C. atrata* to expand even to the cooler northern region (Kim et al. 2014).

In a previous study, we constructed a draft genome of *C. atrata* and selected single-nucleotide polymorphisms (SNPs) after performing whole-genome sequencing (WGS) for about each 10 specimens collected from four South Korean localities: three mainland populations and the Jeju Island population (Park et al. 2025). Among the three mainland populations, Inje is located at a higher elevation in the newly expanded northeastern cooler region in Gangwon Province. In comparison, the remaining two southern mainland populations are more than 300 km south of Inje. Population structure analyses on the basis of ∼48 million SNPs and ∼200,000 missense SNPs, to improve resolution, did not reveal any genetic difference among the three mainland populations. However, more information was provided using the latter approach.

Given the ecologic characteristics of *C. atrata* and the lack of genetic differences among mainland populations, the recent establishment of *C. atrata* in the northeastern region, including Inje, is noteworthy. An adaptation to lower temperature was hypothesized in the previous study; however, this hypothesis was not supported because no structure was detected even when using a substantial number of missense SNPs alongside all 48 million SNPs (Park et al. 2025). Instead, it has been proposed that an extensive dispersal under the rising temperature conditions and subsequent exploration of suitable habitats has allowed *C. atrata* to thrive in the newly expanded region. However, another study, which included additional populations, particularly from newly expanded regions, was conducted to improve understanding of the effect of global warming, particularly regarding the main factor facilitating range expansion.

Moreover, to improve the limitations of our previous genome-wide study and the detection of potential fine-scale genetic differentiation, we conducted a follow-up investigation using expanded geographic sampling and a targeted SNP-based approach. Sampling was strategically designed to include populations from both the warmer southern regions and the cooler northern regions of South Korea, to which *C. atrata* has recently expanded. Indeed, by comparing historically established and recently colonized populations, this study aimed to evaluate whether climatic variation contributes to the emerging genetic structures during range expansion from climate change.

Materials and Methods

Sampling localities and annual temperatures. This study included six mainland populations and one from Jeju Island (Fig. 1). Inje and Hongcheon are located in the northeastern region of Gangwon Province, and Paju is located west of the two northeastern localities at about the same latitude. These three localities are newly expanded regions. The mean annual temperatures for the past 20 yr (from 2001 to 2020) were obtained from the Korea Meteorological Administration (https://www.kma.go.kr/neng/index.do) and are 10.53°C in Inje, 11.10°C in Hongcheon, and 10.98°C in Paju; these are categorized as the lower temperature zones (Table 1). Comparatively, the three other mainland populations are located in the southern regions: Gwangju in the southwestern region and both Ulsan and Busan in the southeastern region (Fig. 1). The mean annual temperatures were 14.27°C in Gwangju, 14.50°C in Ulsan, and 15.0°C in Busan. Moreover, these three southern localities are highly urbanized cities. The remote Jeju Island presented a mean annual temperature of 16.26°C. Thus, the three southern localities and Jeju have substantially higher yearly temperatures than the northern ones and were categorized as the higher temperature zones.

DNA extraction. A total of 110 *C. atrata* adults were collected from the seven localities in 2021 (Table 2). Total DNA extraction was performed from one leg of each *C. atrata* using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI).

SNP selection and primer design for amplicon sequencing. The *C. atrata* genome is approximately ~5.0–5.2 Gb, substantially larger than the typical insect genome size of 1.0 Gb (Park et al. 2025). Therefore, constructing reference genomes and conducting population genetic analyses for such a large genome requires substantial costs and time. In particular, population genetic analyses that use WGS significantly increase data production costs, considering the number of individuals subjected to data production. To overcome these limitations, we conducted amplicon sequencing using the SNPs detected in a previously studied population (Park et al. 2025), targeting

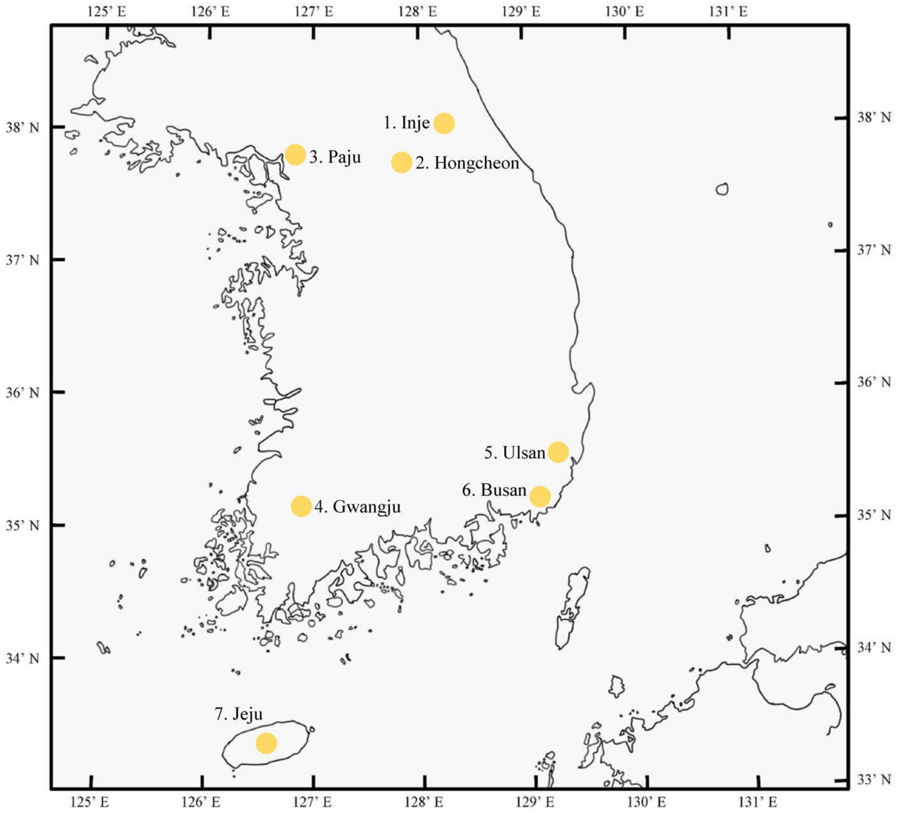

Fig. 1. *Cryptotympana atrata* sampling locations in South Korea. General locality names are provided on the map.

an increased number of populations with an increased sample size. Park et al. (2025) secured 48,772,620 SNPs from 39 individuals, collected from four populations in South Korea through WGS. To reselect the SNPs for amplicon sequencing, the nonsynonymous SNPs and missense SNPs required to secure more conserved variants that could affect protein sequences and induce phenotypic changes were initially filtered, resulting in 119,649 SNPs. Subsequently, we applied data mining techniques, specifically the feature selection using the information gain algorithm, to select SNPs that reflect differences among populations, resulting in the final selection of 239 SNPs for amplicon sequencing. Only 1 primer pair was designed to amplify the neighboring SNPs simultaneously, whereas 188 primer pairs were used for amplicon sequencing. Using these primers, we constructed amplicons for each individual, attached indices for demultiplexing, and built a library suitable for Illumina sequencing. Subsequently, 150 base pairs × 2 paired end data were generated from the constructed library using the Illumina iSeq100 (Illumina Inc., San Diego, CA).

Variant calling. Reads for each sample filtered using Trimmomatic version 0.39 were aligned to the constructed draft genome using bwa-mem2 version 2.1 (Vasimuddin

Table 1. Average annual temperature in the collection localities of *Cryptotympana atrata* for 20 yr.

	Locality						
Year	**Inje**	**Hongcheon**	**Paju**	**Gwangju**	**Ulsan**	**Busan**	**Jeju**
2001	10.5	10.2	—[a]	13.8	14.6	15.3	16.1
2002	10.5	10.2	10.8	13.9	14.4	14.7	15.8
2003	10.1	10.4	10.9	13.6	14.3	14.3	15.7
2004	10.7	10.9	11.0	14.3	15.2	14.9	16.4
2005	10.0	10.3	10.3	13.6	14.3	13.8	16.2
2006	10.6	11.1	11.3	14.2	14.6	14.7	16.2
2007	10.8	11.3	11.3	14.6	15.0	15.3	16.5
2008	10.2	11.0	11.0	14.6	14.2	15.0	16.0
2009	10.6	11.2	11.0	14.6	14.3	15.2	16.0
2010	10.2	10.9	10.6	14.2	14.0	14.9	15.6
2011	9.4	10.7	10.4	13.7	13.7	14.6	15.6
2012	10.0	10.6	10.9	13.7	13.7	14.5	15.7
2013	10.6	10.8	11.0	14.2	14.8	15.3	16.5
2014	11.1	11.7	11.4	14.3	14.7	15.1	16.2
2015	11.1	12.2	11.6	14.6	15.0	15.4	16.7
2016	11.2	12.3	11.7	15.0	14.8	15.7	17.0
2017	10.5	11.3	10.5	14.6	14.5	15.2	16.8
2018	10.1	11.2	10.5	14.6	14.3	15.1	16.6
2019	11.2	12.1	11.3	14.7	14.9	15.7	16.8
2020	11.2	11.6	11.2	14.5	14.6	15.2	16.7
Average temperature	10.53	11.10	10.98	14.27	14.50	15.00	16.26
Standard deviation	0.49	0.64	0.40	0.42	0.41	0.46	0.44
Significance test*	a	b	b	c	c	d	e

[a] —, not available.

* Significance test was conducted using Tukey honestly significant difference *post hoc* test with significance noted at $P \leq 0.05$.

et al. 2019) with default parameters. Polymerase chain reaction duplicates were filtered using the rmdup module in SAMtools version 1.11 (Li et al. 2009), and the genotype for each sample was secured through calling using mpileup in SAMtools and BCFtools version 1.13 with the -mv -Ov parameters. After the raw variant calling, InDel and multiallelic loci were removed, and only variants with a (QV) $>$ 30 were selected using VCFtools

Table 2. Genetic diversity estimates for each population on the basis of 118 loci.

Population	n^a	Number of loci	Number of usable loci	Number of polymorphic loci	Percentage of polymorphic loci
Inje	17	118	118	69	58.47
Hongcheon	14	118	118	68	57.63
Paju	9	118	118	62	52.54
Gwangju	15	118	118	89	75.42
Ulsan	20	118	118	74	62.71
Busan	15	118	118	70	59.32
Jeju	20	118	118	87	73.73
Average	15.71	118	118	74.14	62.831

[a] *n*, number of tested individuals.

version 0.1.16 (Danecek et al. 2011), with -minQ 30, –remove-indels, –max-missing 1.0, –min-alleles 2, and –max-alleles 2 parameters. Annotation of the variants was conducted by constructing a custom database for SnpEff version 5.1 (Cingolani et al. 2012) on the basis of the constructed gene model using the build module in SnpEff.

Genetic diversity and distance. Genetic diversity within the population was measured as the number of alleles (N_a), the number of effective alleles (N_e), observed heterozygosity (H_O), and expected heterozygosity (H_E) using GenAlEx version 6.5 (Peakall and Smouse 2012). In addition, this software was used to determine the F_{IS} for each population (Hartl and Clark 1997), a measure of the heterozygosity deficiency resulting from nonrandom mating. A pairwise estimate of the genetic distance (F_{ST}) was calculated to measure the degree of correlation between population pairs using the infinite allele mutation model with Arlequin version 3.5 (Excoffier and Lischer 2010). In addition, this software was used to calculate the significance of the F_{ST} using the Fisher exact test on the basis of 10,000 permutations. Pairwise F_{ST} was used to estimate N_m (the product of effective population size N_e and migration rate m) according to Excoffier et al. (1992) using the following equilibrium relationship: $F_{\mathrm{ST}} = 1/(4N_m + 1)$. To test for correlation between geographic and genetic distances, a Mantel test was performed (Mantel 1967) using isolation-by-distance (IBD), which compares the matrices of pairwise genetic distance $F_{\mathrm{ST}}/(1 - F_{\mathrm{ST}})$, and the geographic distance data (kilometers), with the significance test conducted over 10,000 randomizations (Mantel 1967). The analysis was performed using the IBD software package version 1.52 (Bohonak 2002).

Population genetic structure. The F_{ST} values were then used for the principal coordinates analysis (PCoA) via covariance, and the genetic distances were standardized to detect and plot the relationships among populations using GenAlEx version 6.5 with default parameters (Peakall and Smouse 2012). STRUCTURE version 2.3.3 was used to assess the most probable number of genetically distinct clusters (K) in the

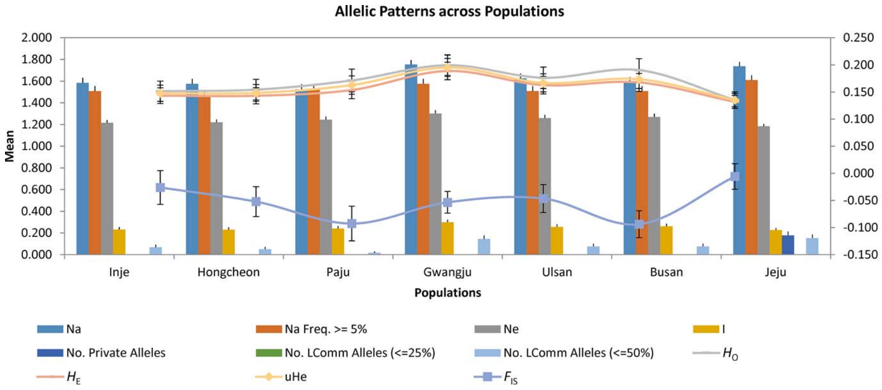

Fig. 2. Allelic patterns across seven populations of *Cryptotympana atrata*. N_a, number of different alleles; N_a (frequency ≥ 5%), number of alleles with frequency greater than 5%; N_e, number of effective alleles; *I*, Shannon's information index; no. private alleles, number of alleles unique to a single population; no. LComm alleles (≤ 25%), number of locally common alleles occurring in 25% or less of the populations; no. LComm alleles (≤ 50%), number of locally common alleles occurring in 50% or less of the populations; H_E, expected heterozygosity; H_O, observed heterozygosity; and F_{IS}, inbreeding coefficient. Vertical bars represent the standard error.

populations (Evanno et al. 2005). An admixture model with correlated allele frequencies was used, with the *K* value ranging from 1 to 10. For each *K* value, 10 independent runs were performed with a burn-in period of 10,000 iterations, followed by 500,000 iterations for data collection. The structure result was visualized using the web-based tool STRUCTURE HARVESTER version 0.6.94 (Earl and vonHoldt 2012).

Results

Genetic diversity. A total of 118 SNPs were identified from the variant calling on the amplified amplicons for 239 SNPs across 110 individuals. The number of polymorphic loci ranged from 62 to 89, averaging 74.14 (Table 2). The allelic patterns across populations showed no obvious difference among populations (Fig. 2). The *Na* per population ranged from 1.525 (Paju, standard error [SE] = 0.046) to 1.737 (Jeju, SE = 0.041); no statistical difference was observed among populations when the SE was considered. The N_e ranged from 1.185 (Jeju, SE = 0.021) to 1.302 (Gwangju, SE = 0.030) without statistical difference. The number of private alleles, which correspond to those unique to a single population, was zero in most populations; however, for Jeju it was 0.178 (SE = 0.035), suggesting that population-specific alleles are uncommon. The within-population gene diversity, which corresponds to H_E in the diploid data, ranged from 0.135 (Jeju, SE = 0.012) to 0.195 (Gwangju, SE = 0.016), with a mean value of 0.161; no statistical difference was noted among populations when the SE was considered. Similarly, H_O ranged from 0.135 (Jeju, SE = 0.015) to 0.199 (Gwangju, SE = 0.019), with a mean value of 0.168, and exhibited no significant differences among populations. All populations presented higher H_O values than H_E, although the difference was not significant after the SE was considered. All populations showed negative F_{IS}, ranging from -0.094 (Busan, SE $= 0.025$) to -0.006 (Jeju, SE $=$ 0.024), indicating no recognizable inbreeding occurs. The F_{IS} values were not substantially different among populations when the SE was considered. Taken together, no population showed an obvious difference in genetic diversity estimates.

Population genetic analyses. The F_{ST} analysis of *C. atrata* populations revealed significant genetic differentiation in two newly expanded northeastern Inje and Hongcheon populations and the southernmost remote Jeju population from all other populations ($P < 0.05$; Table 3). Jeju presented the highest F_{ST} values, ranging from 0.79574 (Gwangju) to 0.82963 (Hongcheon) under the maximum score of 1.0, indicating a clear genetic isolation from all mainland populations. Indeed, N_m, the number of migrants, between Jeju and other populations was far lower than one per generation (0.10313 to 0.12834). Conversely, very low F_{ST} values were found when comparing Inje and Hongcheon to each other at 0.01471 and other mainland populations, from 0.01869 (Hongcheon versus Busan) to 0.5132 (Inje versus Gwangju). However, all these values have the statistical support at $P < 0.05$. Consistently, Inje and Hongcheon showed a higher N_m at 33.5 individuals per generation to each other and substantially higher values to other mainland populations, ranging from 26.3 (Hongcheon versus Busan) to 9.24 (Inje versus Gwangju). Thus, the two northeastern populations exhibited a certain level of gene flow to each other and other mainland populations, even with the statistically significant F_{ST} values.

In contrast, another newly expanded Paju population, which is located in the cooler area in northwestern region, showed genetic connections with all the southern mainland populations (Gwangju, Ulsan, and Busan), presenting very low F_{ST} values, ranging from 0.00829 (Busan) to 0.01246 (Gwangju) without any statistical significance, along with substantially higher N_m values, ranging from 39.6 (Gwangju) to 59.82 (Busan). The geographically closer southeastern Ulsan and Busan populations showed a very low F_{ST} value at -0.00153, corresponding to unlimited gene flow, suggesting a high degree of genetic similarity. Although the Gwangju and Ulsan populations are quite distant, the two populations showed a lower F_{ST} value at 0.00675 without any statistical significance, revealing a higher N_m value at 75.61. Collectively, these results suggest a population genetic pattern that is somewhat inconsistent with geographic distance (Fig. 1) and unreflective of the mean annual temperature that was substantially lower in the three newly expanded northern populations (Table 1).

Given that the Jeju population showed an overwhelming genetic distance to other populations, subsequent analyses were performed with two schemes, not to obscure detailed relationships among the mainland populations: one including Jeju and the other excluding Jeju. The IBD test including Jeju showed a strong IBD at the level of $P < 0.002$ ($R^2 = 0.4971$; Fig. 3A), whereas the analysis excluding Jeju did not show any IBD ($P < 0.121$; $R^2 = 0.0926$; Fig. 3B), indicating that genetic distance among the mainland populations is overall not reflective of geographic distance. Considering the three newly expanded populations are located in the cooler northern region, whereas those of preexisting populations are located in the warmer southern region, with the least distance of 300 km, the lack of IBD among mainland populations further implies that the degree of gene flow among mainland populations is overall not reflective of the lower mean annual temperature.

Table 3. Fixation indices (F_{ST}) and migration rate (N_m) between population pairs of *Cryptotympana atrata*.

	1	**2**	**3**	**4**	**5**	**6**
Inje						
Hongcheon	$F_{ST} = 0.01471^*$					
	$N_m = 33.49697$					
Paju	$F_{ST} = 0.03357^*$	$F_{ST} = 0.03018^*$				
	$N_m = 14.39382$	$N_m = 16.0652$				
Gwangju	$F_{ST} = 0.05132^*$	$F_{ST} = 0.03629^*$	$F_{ST} = 0.01246$			
	$N_m = 9.24238$	$N_m = 13.27683$	$N_m = 39.63453$			
Ulsan	$F_{ST} = 0.03437^*$	$F_{ST} = 0.02068^*$	$F_{ST} = 0.01133$	$F_{ST} = 0.00657$		
	$N_m = 14.04776$	$N_m = 23.68048$	$N_m = 43.61563$	$N_m = 75.61444$		
Busan	$F_{ST} = 0.04140^*$	$F_{ST} = 0.01869^*$	$F_{ST} = 0.00829$	$F_{ST} = 0.01821^*$	$F_{ST} = -0.00153$	
	$N_m = 11.57609$	$N_m = 26.25773$	$N_m = 59.82421$	$N_m = 26.95557$	$N_m =$ infinite	
Jeju	$F_{ST} = 0.82900^*$	$F_{ST} = 0.82963^*$	$F_{ST} = 0.82217^*$	$F_{ST} = 0.79574^*$	$F_{ST} = 0.81354^*$	$F_{ST} = 0.81148^*$
	$N_m = 0.10313$	$N_m = 0.10268$	$N_m = 0.10814$	$N_m = 0.12834$	$N_m = 0.1146$	$N_m = 0.11616$

* $P < 0.05$.

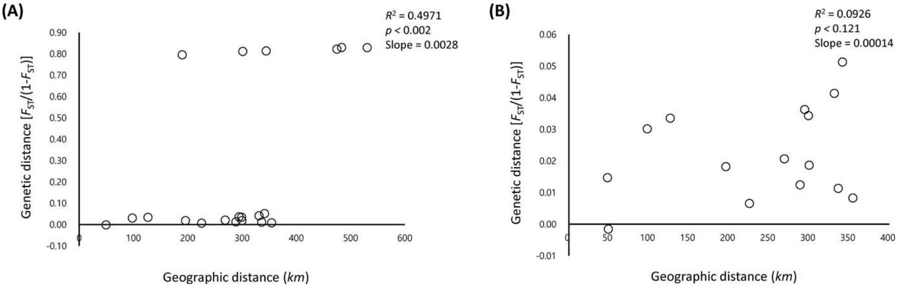

Fig. 3. Isolation by distance that was assessed by plotting $F_{ST}/1 - F_{ST}$ against geographic distance, along with the corresponding coefficient of determination (R^2), P value, and slope of the regression line for data from 118 SNPs. (A) Analysis including Jeju and (B) analysis excluding Jeju.

The PCoA also was performed in two schemes (Fig. 4). The analysis including Jeju showed that the first principal coordinates of individual genotypes accounted for the majority of the variance at 98.27%, whereas the second principal coordinates explained only 1.00% of the variance (Fig. 4A). Jeju alone formed a highly divergent group mainly by the first principal coordinates, as was estimated in the F_{ST} analysis. In contrast, the other mainland populations, including the Paju population, did not show any divergence by the first component, which accounts for most genetic variance (Table 3). Two northeastern Inje and Hongcheon populations were slightly separated from the remaining mainland populations; however, this separation accounted only for only 1% of the variance (Fig. 4A). The analysis excluding Jeju showed that the Inje and Hongcheon populations formed a somewhat divergent group (Group A), separated from the remaining mainland populations (Group B) mainly by the first principal coordinates, along with a slight divergence between the Gwangju and Paju subgroup and the Ulsan and Busan subgroup by the second component (Fig. 4B). Considering the extent of the first principal coordinates, which accounted for 59.69%, this analysis

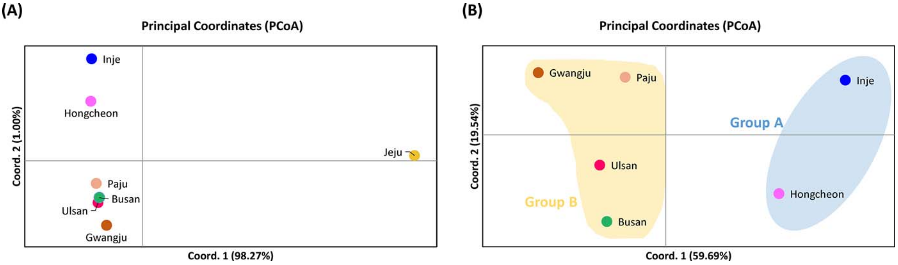

Fig. 4. Results of the principal coordinates analysis (PCoA) depicting allelic variance in *Cryptotympana atrata*. (A) A total of 110 individuals were included from seven populations, including Jeju. (B) A total of 90 individuals from six populations, excluding Jeju. The percentage variation explained by the first and second axes is indicated.

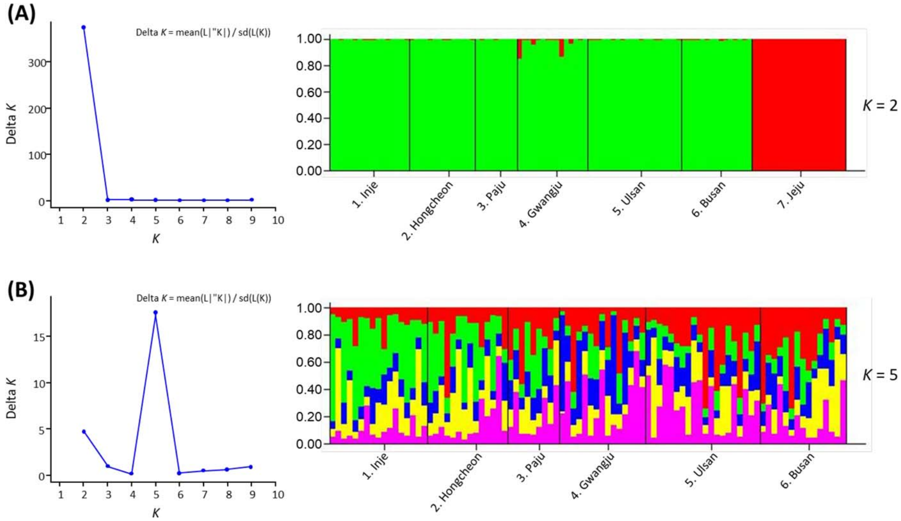

Fig. 5. Clustering data analysis for 118 SNPs in *Cryptotympana atrata* that was performed using STRUCTURE software. The delta *K* plot was calculated using the formula delta *K* = mean (|L″(*K*)|)/sd(L(*K*)). (A) Clustering analysis of seven populations, including Jeju, in *K* = 2. (B) Clustering analysis of six populations, excluding Jeju, in *K* = 5. Black bars separate populations. Different colors represent different gene pools.

explains the presence of a certain level of genetic divergence in the two northeastern populations from the remaining ones, as was detected in F_{ST} analysis (Table 3). On the other hand, the genetic divergence between the Gwangju and Paju subgroups and the Ulsan and Busan subgroups does not appear substantial, with only 19.54% of the variance found for this separation. Meanwhile, no absolute genetic isolation between the two subgroups was observed in the F_{ST} analysis (Table 3).

To investigate the population structure and admixture patterns among *C. atrata* populations, STRUCTURE analysis was conducted for the two schemes (Fig. 5). An examination of the likelihood scores from 10 replicate runs across *K* values ranging from 1 to 10 indicated that $K = 2$ was the most likely number of distinct gene pools when the Jeju population was included; meanwhile, $K = 5$ was the most likely when Jeju was excluded. The inclusion of Jeju indicated that all the mainland populations have only one gene pool (green) without any structure, whereas Jeju has a completely different gene pool (red), depicting an absence of genetic structure among all mainland populations as a whole (Fig. 5A). Gwangju also exhibited a small portion of the red gene pool, which was found exclusively in Jeju, indicating a small but minimal portion of genetic interchange between the two populations. Alternatively, the STRUCTURE analysis, excluding Jeju, showed no discernible gene pool among populations (Fig. 5B), indicating that inland populations have not been structured during range expansion. Nonetheless, the gene pool composition differed slightly among populations (Fig. 5B). Roughly, Inje and Hongcheon had relatively higher proportions of the green and yellow gene pools, whereas Paju, Gwangju, Ulsan, and Busan had higher proportions of the purple, red, and blue gene pools, indicating a minimal level of compositional difference between the Inje and Hongcheon subgroups and the remaining mainland populations.

Discussion

This study analyzed the genetic diversity and structure of *C. atrata* populations across the South Korean mainland and the remote Jeju Island using 118 SNPs identified from direct amplicon sequencing. Notably, no significant difference in genetic diversity among populations was observed (Table 2); meanwhile, a lack of IBD (excluding Jeju) and higher H_O values than H_E, and negative values of F_{IS} were also discovered (Fig. 3; Table 2). The population genetic structure analyses presented two notable results, such as a slight genetic distinction in two of the three newly expanded populations (Inje and Hongcheon) located in the cooler regions from other mainland populations (Table 3; Figs. 4B, 5B) and a significant genetic isolation of Jeju from the mainland populations (Table 3; Figs. 4A, 5A). Other minor, but notable results included the discovered genetic similarity between the two geographically distant Paju and Gwangju populations (Table 3; Fig. 4B) and the small portion of the shared gene pool between the southeastern Gwangju population and the one from the remote Jeju Island (Fig. 5B).

Genetic relationships among mainland populations. Considering several ecologic features of *C. atrata*, including sensitivity to temperature variation, a short dispersal distance, and a high fidelity to emergence sites (Karban 1981, Ki et al. 2016, Simões and Quartau 2007, Xie et al. 2023), we, in the early phase of the study, hypothesized that the expansion of *C. atrata* populations to the cooler northern region could be associated with an increase in adaptive traits, which are related to lower temperatures, because the temperature in the expanded region is substantially lower than in the southern locations in South Korea (Table 1). To confirm possible influential factors, this study included three newly expanded northern populations, which are located in the cooler region with a similar latitude (∼38°N) and mean annual temperature (10.53–11.10°C), along with three southern mainland populations with a similar latitude (∼35.5°N) and obviously higher temperature (14.27–15.0°C; Table 1; Fig. 1). Unexpectedly, we found that, excluding northwestern Paju, only northeastern Hongcheon and Inje exhibited a slight genetic distinction from the remaining mainland populations (Table 3; Figs. 4B, 5B). These findings contrast with previous findings in which no distinction was observed in Inje (Park et al. 2025). Conversely, Paju showed a high genetic similarity to Gwangju, which is located ∼350 km away in the warmer southern region (Table 3; Fig. 4B). Thus, the current result explains more clearly that the expansion of *C. atrata* to the northern region is not associated solely with adaptive traits to these lower temperatures. Considering that the 238 SNPs used in this study are nonsynonymous and missense, possibly including those that induce adaptive traits and protein sequence changes, no supportive data were obtained for the positive relationship between range expansion to low temperature zones and mean annual temperature. Thus, the current result requires an alternative explanation for the expansion of *C. atrata* into the northern region.

Note that the newly expanded leading edge may not yet be suitable enough to establish new populations due to a heterogeneous environment (Woolbright et al. 2014). Consequently, genetic isolation from core populations and reduced genetic diversity have often been predicted in the newly expanded regions (Woolbright et al. 2014). A consistent result of this prediction could be a slight genetic distinction in the two northeastern populations, Inje and Hongcheon, from the remaining populations (Table 3; Figs. 4B, 5B). However, the degree of isolation was only slight, and another expanded population, Paju, did not show any isolation, even exhibiting a closer genetic relationship to the distant, southern Gwangju population (Table 3; Figs. 4B, 5B). Moreover, the two northeastern populations, along with the other studied populations presented a higher H_O than H_E, negative values of F_{IS}, and lack of IBD (excluding Jeju), with the similar genetic diversity estimates (Table 2; Figs. 2, 3B), which contrasts with the general expectation at the newly expanded regions. These results strongly signal that the two northeastern populations and other studied populations have certain population sizes, without suffering from inbreeding and genetic drift, which are typically detectable in small, isolated populations. This finding could be interpreted as the consequence that the rising temperature in traditionally cooler regions, such as Gangwon Province, may have strongly facilitated to create some suitable microhabitats that allow *C. atrata* to explore and thrive extensively within Inje and Hongcheon, even though overall environmental conditions, including temperature, in the area remain suboptimal. Considering the recent population expansion, more time could still be required for the two northeastern populations to become more genetically similar to other mainland populations, overcoming slightly limited genetic exchange. Indeed, a simulation study suggested that expansion to new habitats will promote the founder effect; however, continual formation in new areas will ensure the new habitat has a genetic composition comparable with core areas (McInerny et al. 2009). These findings and our inference collectively suggest that global warming at present does not contribute overall to the equivalent environmental conditions for the preexisting and the newly expanded regions; however, the impact of global warming seems to be substantial enough for the *C. atrata* populations to sustain and thrive at least locally in the newly expanded regions.

Genetic isolation in the Jeju Island population. Even though *C. atrata* in South Korea has long been distributed abundantly both in Jeju Island and southern inland regions (Kim and Song 2017, Sun et al. 2018), all datasets consistently present evidence of a strong genetic isolation between them (Table 3; Figs. 2, 4A, and 5A). This pronounced genetic divergence can be interpreted as evidence of the geographic isolation on Jeju Island, rather than global warming. During the last glacial maximum, approximately 20,000 yr before present (YBP), Jeju Island was connected to the Korean Peninsula due to lowered sea levels; however, it became isolated around 12,000 YBP (Ohshima 1990). Therefore, the genetic independence of the Jeju population likely reflects a cumulative genetic divergence over the subsequent 12,000 yr. This result was also evidenced in the previous study, which used a substantial number of *C. atrata* SNPs (Park et al. 2025).

Despite the nearly complete isolation of the Jeju population from the mainland populations, the Jeju population presented the highest F_{ST}, whereas the PCoA illustrated an isolation, and the STRUCTURE analysis identified the possession of a unique gene pool (Table 3; Figs. 4A, 5A). Moreover, the STRUCTURE analysis presented evidence of a minimal genetic relationship between the Jeju and the inland Gwangju populations, which shared the red gene pool (Fig. 5A). This slight genetic connectivity may have occurred through various ways, for example, through an increased movement of goods via air and seaport facilities, human transportation, and natural phenomena, including typhoons (Bullock et al. 2018). Busan, the major port city in South Korea, represents the predominant transportation hub for passengers and cargo between Jeju and the mainland; however, no notable genetic exchange was detected here (Fig. 5A). Thus, the observed genetic connectivity between Jeju Island and Gwangju is likely facilitated by other methods, instead of human-facilitated dispersal. Indeed, South Korea experiences three to four typhoons annually during the summer, the peak season for *C. atrata* activity (Cha et al. 2009). Most of these typhoons originate from outside South Korea and travel more immediately toward the southwestern regions of the Korean Peninsula, including Gwangju via Jeju Island (Kim et al. 2020). Thus, such passive dispersal is likely a source of gene pool transfer from Jeju to Gwangju.

In conclusion, current population genetic analysis of *C. atrata* using SNPs indicated that global warming allowed *C. atrata* to expand to previously unoccupied cooler inland regions, such as Inje, Hongcheon, and Paju; meanwhile, only a slight genetic isolation was observed in the two northeastern regions of Inje and Hongcheon. The main factor that facilitated the expansion to new areas appears not to be an adaptation to lower temperatures. Instead, global warming seems to have facilitated dispersal, exploration of microhabitats, and success, even under the unfavorable lower temperature conditions. A strong genetic isolation of the Jeju Island population indicated that the trace of biogeographic history is more obviously reflected than ongoing global warming. Therefore, the current study exemplifies that the major factors that govern the genetic structure of the current population differ in each inland and island area in South Korea under global warming, requiring a cautious interpretation of the major factors.

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