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Population genetic structure and putative migration pathway of *Sogatella furcifera* (Horváth) (Hemiptera, Delphacidae) in Asia

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Abstract

The white-backed planthopper, *Sogatella furcifera* (Horváth) (Hemiptera, Delphacidae), has emerged as a serious rice pest in Asia. In the present study, 12 microsatellite markers were employed to investigate the genetic structure, diversity and migration route of 43 populations sampled from seven Asian countries (Bangladesh, China, Korea, Laos, Nepal, Thailand, and Vietnam). According to the isolation by distance analysis, a significant positive correlation was observed between genetic and geographic distances by the Mantel test ($r^2 = 0.4585$, $P = 0.01$), indicating the role of geographic isolation in the genetic structure of *S. furcifera*. A population assignment test using the first-generation migrants detection method (thresholds $a = 0.01$) revealed southern China and northern Vietnam as the main sources of *S. furcifera* in Korea. Nepal and Bangladesh might be additional potential sources via interconnection with Vietnam populations. This paper provides useful data for the migration route and origin of *S. furcifera* in Korea and will contribute to planthopper resistance management.

Keywords: *Sogatella furcifera*, microsatellite, population genetics, migration pathway

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Introduction

The white-backed planthopper, *Sogatella furcifera* (Horváth) (Hemiptera, Delphacidae), is a long-range migrant and is a major rice insect pest in Asia (Kisimoto & Sogawa, 1995; Sogawa, 2014). As Chinese hybrid rice varieties have spread throughout China and Vietnam, outbreaks of *S. furcifera* have occurred subsequently in these regions. Furthermore, *S. furcifera* has become problematic in Japan (Sogawa, 2014). In temperate Asian countries, including Korea, Japan, and northern China, *S. furcifera* cannot overwinter and is thought to migrate from northern Vietnam or southern China in June and July (Matsumoto *et al.*, 2013). *S. furcifera* overwinters in northern Vietnam, and migrates to southern China from April to early May (Otuka *et al.*, 2008; Hu *et al.*, 2017), and is also able to overwinter in southern China (Hu *et al.*, 1988, 2015). Therefore, populations of *S. furcifera* in southern China are likely to be mixed. It is unknown whether northern Vietnam populations are homogeneous or mixed. The sources of *S. furcifera* populations in temperate Asian countries, in particular Korea and Japan, remain unclear.

To elucidate the source regions of *S. furcifera* in these regions, the population structure of *S. furcifera* has been examined with various molecular markers, which include cytochrome c oxidase subunit I (COI), inter-simple sequence repeats (ISSR), and microsatellites. Mun *et al.* (1999) found

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that COI sequence variation of *S. furcifera* was low in Korea, China, Malaysia, Philippines, and Vietnam. Matsumoto *et al.* (2013) used *cox1–trnL2-cox2* data to examine *S. furcifera* populations from ten Asian regions (Japan, China, Taiwan, northern and southern Vietnam, Laos, Thailand, northern and southern Philippines, and Papua New Guinea) and found little genetic differentiation among these Asian populations. In contrast, Liu *et al.* (2010) found a high level of genetic variation among populations of *S. furcifera* in China, Laos, Myanmar, and Vietnam by ISSR analysis. The *S. furcifera* populations were grouped into two clusters; one from northern Vietnam and Laos to southeastern and central Yunnan, and the other from Myanmar to southwestern Yunnan. Sun *et al.* (2014) examined genetic variation of five *S. furcifera* populations 1314 km apart in southern China using 19 microsatellite markers. The level of population differentiation was very low, forming one genetic cluster.

In general, population genetic studies on *S. furcifera* in Asian countries (Mun *et al.*, 1999; Matsumoto *et al.*, 2013; Sun *et al.*, 2014; Qiu *et al.*, 2016) show high levels of genetic connectivity and strong gene flow in *S. furcifera*, which makes it difficult to identify the exact sources of *S. furcifera* in temperate Asian countries. Northern Vietnam or southern China are considered the primary sources for the Far East Asian populations. While there is a possibility that all the Asian populations of *S. furcifera* may be one panmictic population, it is still very important to more specifically elucidate migration patterns, gene flow, and genetic connectivity among various geographic populations of *S. furcifera* in Asia because characterization of biotypes, such as insecticide resistance and susceptibility to resistant rice varieties, is crucial for planthopper management (Matsumoto *et al.*, 2013).

In the present study, we sampled *S. furcifera* from 43 locations in seven countries in Asia (Korea, Bangladesh, China, Laos, Nepal, Thailand, and Vietnam). Also, using the microsatellites described by Nam *et al.* (2015), we characterized the genetic differentiation and gene flow among these populations, and identified possible migration routes of *S. furcifera* to Korea.

Materials and methods

Insect samples

S. furcifera was collected in 2012, 2013, and 2014 from various countries in Asia (Supplementary Table S1). These comprised one site each in Laos, Korea, Nepal, Thailand, and Vietnam, four sites in Bangladesh in 2012; one site each in China, Nepal, and Thailand, two sites in Bangladesh, and 15 sites in Korea in 2013; and four sites in China and ten sites in Korea in 2014. Samples from Bangladesh, Vietnam, Thailand, Nepal, and Laos were collected as part of the Asian Food and Agriculture Cooperation Initiative and other samples were collected by us. All the samples were collected using sweeping net and put in vials with 95% ethanol and stored at −20°C until DNA extraction.

Microsatellite genotyping

DNA was extracted from individuals without abdomen using the AccuPrep DNA Extraction Kit (Bioneer, Korea). For genotyping, we used 12 microsatellite loci developed for *S. furcifera* by Nam *et al.* (2015). Polymerase chain reaction (PCR) was performed in two separate multiplex groups. Multiplex group 1 included WBPH_T5, WBPH_T7, WBPH_T9, WBPH_T11, WBPH_T13, and WBPH_T16 markers. Multiplex group 2 included WBPH_T3, WBPH_T4, WBPH_T7, WBPH_T8, WBPH_T15, and WBPH_T18 markers. We used Takara Taq ™ (TaKaRa, Japan) in a total volume of 10 µl, with 4.9 µl distilled water, 1.0 µl 10 × PCR buffer, 1.0 µl 10 mM dNTP mixture, 0.5 µl of each primer (final concentration, 10 pmol μl^{-1}), 0.1 µl of Taq polymerase, and 2.0 µl template DNA. PCR was conducted using an initial denaturation of 4 min at 94°C, followed by 35 cycles of 94°C for 30 s, annealing at 61°C for 30 s, 72°C for 40 s, and a final extension at 72°C for 15 min.

Data analysis

Genetic variation and genetic structure

The microsatellite genotype data of *S. furcifera* were analyzed by Micro-Checker (Van Oosterhout *et al.*, 2004) for the presence of null alleles, short allele dominance, and the appearance of non-functional gene regions. The Microsatellite Toolkit (Park, 2001) was used to estimate the mean number of alleles (N_A) per locus, and the observed (H_O) and expected (H_E) heterozygosities. The inbreeding coefficient at each locus, F_{IS}, was calculated using FSTAT v. 2.9.3 (Goudet, 2001). Deviations from the Hardy–Weinberg equilibrium (HWE) were tested by Genepop v. 4.2.1 (Raymond & Rousset, 1995).

The population structure of *S. furcifera* was estimated by the Bayesian clustering procedure using STRUCTURE 2.3.3 (Pritchard *et al.*, 2000). As different runs can produce different likelihood values, ten runs were conducted in order to quantify the amount of variation of the most likely number for each *K*. The range of possible clusters (*K*) tested was set from one to ten, with ten iterations. The lengths of the Markov Chain Monte Carlo iterations and burn-in were set to 100,000 and 200,000, respectively. For the *K*-value, STRUCTURE estimates the maximal value of the log likelihood (ln Pr(X/K)) of the posterior probability of each *K*-value (Pritchard *et al.*, 2000). We calculated the mean posterior probability for each *K* value and the second-order rate of change in the log probability of the data between successive values of $\triangle K$, the true number of populations, was calculated by using the program structure harvester (Earl & vonHoldt, 2012).

We conducted an analysis of molecular variance (AMOVA) for the hierarchical partitioning of genetic variation among and within populations. Using the Genalex program (Peakall & Smouse, 2006), we conducted a principal coordinate analysis (PCoA) and then plotted the scatter diagram based on factor scores along the two PCoA revealing most variation. The PCoA analysis was conducted separately for each year.

Gene flow measures

The historical rates of gene flow between populations (N_em) were calculated according to the relationship $N_em = (1 - F_{ST})/4F_{ST}$ (Wright, 1931). N_em infers the effective number of migrants per generation, N_e is the effective population size, and m is the migrant rate. Pairwise estimates of genetic differentiation (F_{ST}) between populations were calculated using FSTAT v. 2.9.3 (Goudet, 2001). Moreover, FreeNA (Chapuis & Estoup, 2007) was used to estimate F_{ST}, considering null alleles, and the results of F_{ST} adjusted for null alleles and F_{ST} assuming no null alleles were compared.

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We plotted $F_{ST}/(1 - F_{ST})$ values against geographic distances between all pairs of sample locations to examine isolation by distance (IBD) using the Mantel test and the Genalex program. By using AMOVA for populations and individuals, the hierarchical partitioning of genetic variation was analyzed, which provided an estimate of the proportion of genetic variation within and between populations.

To detect genetic signatures of dispersal and immigration of *S. furcifera* (Rannala & Mountain, 1997), we conducted population assignment/exclusion tests using the Geneclass2 program (Piry *et al.*, 2004). The direct assignment test (without *P*-value) calculates the proportion of individuals correctly allocated to the most probable population of origin, even if the true population of origin is not clear among the reference populations. The exclusion test calculates the likelihood of a genotype occurring in the population, which is compared with the distribution of likelihoods of simulated genotypes for each reference population. If the likelihood (α) of an individual is below a predetermined threshold (e.g., $\alpha = 0.01$), the population is excluded as the possible origin of the individual (Cornuet *et al.*, 1999). The exclusion method does not presume that the true population of origin has been sampled because this method does not focus on the specific task of immigrant identification and each population is treated independently (Cornuet *et al.*, 1999). In the exclusion test, the frequency possibilities of multilocus genotypes in each reference population were determined using Monte Carlo simulations of 10,000 individuals for the population (Paetkau *et al.*, 2004). We followed a Bayesian statistical approach (Rannala & Mountain, 1997) using the Monte Carlo resampling method.

To infer the migration pathway of individuals between populations, we applied the *detection of first-generation migrants* in Geneclass2 (Piry *et al.*, 2004). We computed the statistical criterion (the ratio L_{home}/L_{max}) for likelihood estimation of migrant detection (*L*) (Paetkau *et al.*, 2004). The assignment criterion values of the simulated independent individuals were computed using a simulation of 10,000 independent individuals at probability thresholds of $\alpha = 0.05$ and $\alpha = 0.01$.

Results

Genetic variability

A total of 118 alleles were identified across the 12 microsatellite loci for 1644 *S. furcifera* individuals from the 43 locations in Asia. Allelic richness varied from 4.855 to 8.482, and H_O and H_E ranged from 0.275 to 0.615 and from 0.506 to 0.790, respectively (table 1). All 43 populations had positive F_{IS} values across loci.

Genetic structure

The genetic differentiation between each pair of populations (corrected pairwise F_{ST}) and the effective number of migrants exchanged per generation (N_em) are shown in Supplementary Tables S2–S4. Uncorrected estimates of pairwise F_{ST} values ranged from 0.0528 for the Bangladesh (B3) and Laos (LA) populations (ENA corrected $F_{ST} = 0.0555$; B3 and LA populations) to 0.2477 for the Vietnam (V) and Thailand (TH) populations (ENA corrected $F_{ST} = 0.2513$; V and TH populations) (Supplementary Table S2). Uncorrected estimates of pairwise F_{ST} values ranged from 0.0400 for the Korea (CN) and Korea (MY) populations (ENA corrected $F_{ST} = 0.0434$; CN and MY populations) to 0.2558 for the Korea (WD) and Korea (CG) populations (ENA corrected $F_{ST} = 0.2489$; WD and CG populations) (Supplementary Table S3). Uncorrected estimates of pairwise F_{ST} values ranged from 0.0088 for the Korea (SA3) and Korea (CW) populations (ENA corrected $F_{ST} = 0.0114$; SA3 and CW populations) to 0.3492 for the Korea (BA) and Korea (MY) populations (ENA corrected $F_{ST} = 0.3222$; WD and CG populations) (Supplementary Table S4). Both estimates of F_{ST} (F_{ST} adjusted for null alleles and F_{ST} assuming no null allele results) were similar. Global estimates of F_{ST} across all loci and all populations were high and significant (uncorrected $F_{ST} = 0.1762$, 95% confidence interval (CI) = 0.1387–0.2137; ENA corrected $F_{ST} = 0.1610$, 95% CI = 0.1285–0.1944). In 2012, the N_em calculated from the uncorrected F_{ST} ranged from 0.759 (Vietnam (V) and Thailand (TH)) to 4.4848 (Laos (LA) and Bangladesh (B3)), and in 2013, N_em ranged from 0.7273 (Korea (CG) and Korea (WD)) to 6.000 (Korea (MY) and Korea (CN)). In 2014, N_em ranged from 0.8017 (China (CH4) and Korea (MY)) to 28.2530 (Korea (CW) to Korea (SA3)). The range of N_em implied an intermediate to high level of gene flow.

AMOVA among *S. furcifera* populations from 43 locations in Asia manifested that most genetic variation was partitioned among populations and individuals within populations (table 2). Approximately 76% of the total genetic variation was accounted for by the individuals within populations, and 24% of the total genetic variation was among populations. Significant correlation was found between genetic and geographical distance among the populations using the Mantel test of IBD ($r^2 = 0.4585$, $P = 0.01$), indicating the role of geographic isolation in the genetic structure of *S. furcifera* (fig. 1).

Results of PCoA analysis are shown in fig. 2. Total variance explained by the first two factors in 2012, 2013, and 2014 were 48% (25.8% for axis 1 and 22.2% for axis 2), 37% (21.5% for axis 1 and 15.8% for axis 2), and 64% (40.9% for axis 1 and 23.1% for axis 2), respectively. In 2012, the population from Thailand (TH) was conspicuously divergent from the others, and in 2014, the population from Milyangshi (MY) in Korea was divergent from the others. In 2013, dispersion of all the population was observed.

Bayesian clustering indicated three clusters ($K = 3$); structure output revealed a maximum value of ΔK of 79.54 (Supplementary Fig. S1). The results of a Bayesian cluster analysis of multilocus microsatellite genotypes in 2012, 2013, and 2014 are displayed as pie graphs in figs 3–5, respectively.

Assignment/exclusion test and investigation of first-generation immigrants

The percentage of *S. furcifera* individuals sampled from each population assigned to and excluded from each reference population, and the mean assignment log-likelihood for each potential donor population in each year, are presented in Supplementary Tables S5–S7. Populations from most locations included members whose possible origins in other populations could be excluded with ≥99% certainty. In Supplementary Tables S5–S7, individuals from all populations could not be assigned to their own population, since the percentage was too low. Among the rest of the population, individuals from the BA (Korea) population in 2013 showed the highest result of percentage, which could be assigned to their own population with 26.3%. In the exclusion test, B1 (2012) population could be excluded with 100% and MY (2014) population could be excluded with 94.7–100% certainty (0.01 threshold) as a putative origin of all populations. The

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Table 1. Genetic variation estimates of geographic populations of *S. furcifera*.

Country	Population	Allelic richness	Sample size	No. of alleles	H_O	H_E	*P*-value[1]	F_{IS}
Bangladesh	B1_12	5.858	40	6.42	0.290	0.599	0.00012	0.526
	B2_12	6.823	37	7.25	0.340	0.667	0.00012	0.500
	B3_12	7.750	41	8.33	0.459	0.773	0.00012	0.416
	B4_12	7.655	39	8.25	0.485	0.691	0.00012	0.310
Vietnam	V_12	6.689	40	7.42	0.606	0.628	0.00012	0.047
Thailand	TH_12	6.596	40	7.17	0.429	0.630	0.00012	0.331
Nepal	NE_12	6.359	39	6.92	0.538	0.643	0.00012	0.175
Korea	SA1_12	7.375	40	7.92	0.615	0.756	0.00012	0.199
Laos	LA_12	7.784	39	8.42	0.594	0.733	0.00012	0.203
Korea	JC_13	6.014	40	6.58	0.427	0.634	0.00012	0.337
	TA_13	8.200	39	8.58	0.494	0.790	0.00012	0.386
	BA_13	7.103	38	7.58	0.368	0.710	0.00012	0.492
	JS_13	6.156	40	6.67	0.323	0.615	0.00012	0.485
	GR_13	7.028	40	7.67	0.306	0.644	0.00012	0.533
	JD_13	6.609	40	7.17	0.288	0.637	0.00012	0.558
	SA213	7.184	40	7.83	0.463	0.672	0.00012	0.323
	WD_13	6.267	40	6.75	0.275	0.574	0.00012	0.530
	CW_13	7.095	40	8.42	0.535	0.674	0.00012	0.218
	KP_13	6.577	40	7.58	0.302	0.667	0.00012	0.556
	NYJ_13	6.587	40	7.17	0.277	0.591	0.00012	0.540
	CG_13	7.096	40	7.17	0.381	0.630	0.00012	0.406
	CN_13	6.309	40	7.50	0.469	0.731	0.00012	0.369
	GS_13	6.956	40	7.00	0.594	0.645	0.00012	0.092
	MY_13	6.723	40	7.33	0.475	0.704	0.00012	0.337
Bangladesh	B5_13	7.418	40	7.50	0.521	0.644	0.00012	0.203
	B6_13	5.818	40	7.75	0.444	0.740	0.00012	0.411
China	CH_13	7.429	40	6.25	0.550	0.647	0.00012	0.162
Nepal	NE_13	6.676	40	8.17	0.531	0.704	0.00012	0.257
Thailand	TH_13	4.940	40	7.17	0.408	0.677	0.00012	0.407
Korea	CW_14	6.161	34	7.42	0.512	0.684	0.00012	0.271
	GS_14	6.967	31	6.08	0.446	0.559	0.00012	0.220
	BA_14	5.779	29	5.08	0.318	0.508	0.00012	0.384
	JC_14	5.681	34	5.33	0.446	0.516	0.00012	0.154
	KP_14	6.484	38	5.08	0.447	0.506	0.00012	0.123
	MY_14	5.952	27	5.42	0.492	0.541	0.00012	0.101
	NYJ_14	4.963	35	6.33	0.380	0.626	0.00012	0.409
	SA3_14	4.855	34	6.58	0.456	0.659	0.00012	0.323
	TA_14	5.341	34	6.00	0.417	0.613	0.00012	0.338
	WD_14	6.001	34	5.58	0.441	0.544	0.00012	0.202
China	CH1_14	6.189	40	6.83	0.471	0.607	0.00012	0.240
	CH2_14	5.686	40	6.50	0.373	0.553	0.00012	0.338
	CH3_14	5.191	40	7.42	0.408	0.641	0.00012	0.374
	CH4_14	8.482	42	6.58	0.434	0.598	0.00012	0.288
Across loci				7.03	0.438	0.642		0.327

Number of alleles, expected heterozygosity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}) and probability (*P*-value) of being in HWE.
[1]Hardy–Weinberg exact test (Raymond & Rousset, 1995) with Bonferroni correction ($P = 0.000123$).

mean assignment likelihood for individuals in 2012 showed that the highest value of the Nepal (Ne) population came from the Laos (La) population (mean assignment log-likelihood = −18.02), while the highest assignment likelihood of the Laos population came from the Bangladesh (B3) (−21.48). Also, the highest assignment likelihood of individuals of the B3 came from the La (−19.75) (Supplementary Table S5). In the mean assignment likelihood for individuals in 2013, the Korea population of JC&CW, WD&JD, BA&CG, GR&KP, and Bangladesh (B5) and Nepal (NE) population showed connection between sites and they were most likely from the same source (Supplementary Table S6). In 2014, the mean assignment likelihood of individuals of the SA3 (Korea) population (except from itself) came from the CH1 (China)

Table 2. AMOVA among *S. furcifera* samples from 43 sites in Asia.

Source of variation	df.	Sums of squares	Mean sums of squares	Estimated variance	% of variation
Among populations	42	5812.589	138.395	3.344	24
Individuals within populations	1601	16,945.402	10.584	10.584	76
Total	1643	22,757.990		13.928	100

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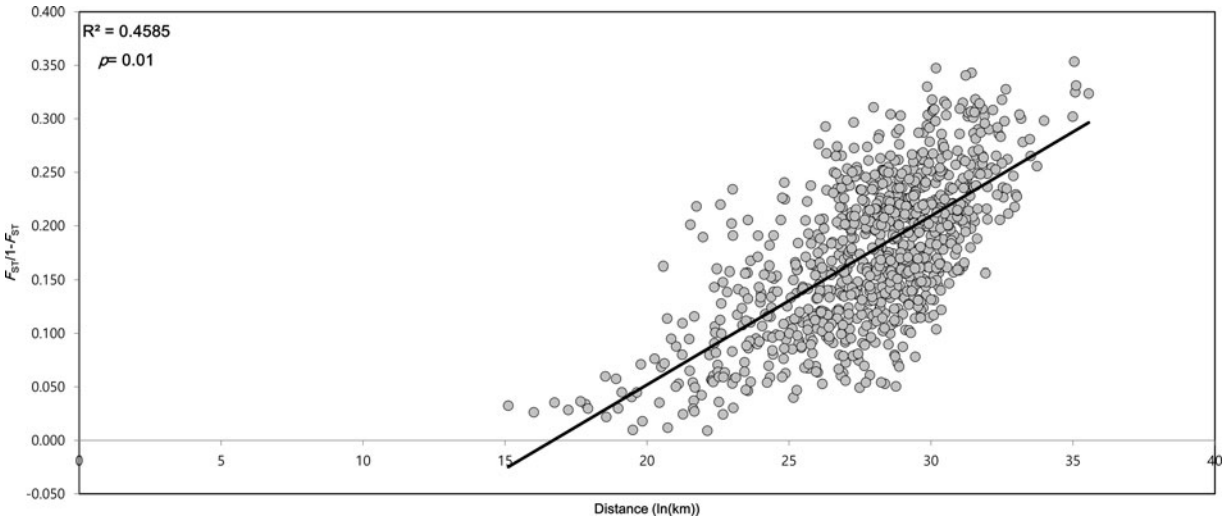

Fig. 1. Geographical distance vs. genetic distance ($F_{ST}/1 - F_{ST}$) for populations of *S. furcifera* in 2012, 2013, and 2014 using pairwise F_{ST}. Correlations and probabilities were measured from a Mantel test with 10,000 bootstrap replicates.

population (−14.26), the population of SA3 and CH1 was most likely from the same source. Moreover, there were connections between TA (Korea) population and CH3 (China), NYJ (Korea) population and CH2 (China), in which China was expected to be a possible source of Korea population (Supplementary Table S7).

The number of first-generation migrant individuals for each year is summarized in Supplementary Tables S8–S10. We considered the L_{home}/L_{max} ratio as for the detection of first-generation immigrant between sample locations. In 2012, a total of 63 and 30 individuals were observed as possible first-generation immigrants at thresholds of $\alpha = 0.05$ and $\alpha = 0.01$, respectively. In 2013, 184 and 82 individuals were identified, respectively, and 81 and 39 individuals were verified, respectively, in 2014 as potential first-generation immigrants. The probable dispersal pathway among populations is displayed in figs 6–8, and was based on the first-generation immigrants at a threshold $\alpha = 0.01$. We eliminated the geographical barrier and reverse movement from Korea. We speculate that there may be several migration pathways of *S. furcifera* to Korea. One putative route is from Nepal (NE) to Northern Vietnam (V) and from Northern Vietnam directly to Southwestern Korea (SA) (e.g., in 2012). In fig. 6, various migration pathways were revealed among Vietnam, Nepal, and Bangladesh, and these relations may sustain the result of direct dispersal pathway from Nepal to Southwestern Korea. The second putative route is from Southern China to Korea (e.g., in 2013 and 2014). It would be also possible that *S. furcifera* would migrate from Northern Vietnam to Korea via Southern China. Moreover, there were frequent migrations of *S. furcifera* among Bangladesh, Laos, and Nepal. It would be plausible that high dispersal rate may show in Southeast and Southwest Asia. In figs 7 and 8, various dispersal pathways are indicated from China to Korea, with a definite genetic connection among them. We verified the movement in Korea individually for 2013 and 2014, high rate of dispersion occurred and movement to adjacent locations appeared among the sites in Korea (Supplementary Fig. S2).

Discussion

In this study, we investigated the genetic diversity and connectivity of *S. furcifera* populations in various locations in Asia. Low level of expected heterozygosity and high level of gene flow (N_em) were observed in these regions. *S. furcifera* has been speculated to migrate from the Indo-China Peninsula, in particular northern Vietnam to China (Sun *et al.*, 2014), and its migration to Korea and Japan is more likely from southern and/or south-eastern China (Mun *et al.*, 1999; Otuka *et al.*, 2008). This long-range migration may cause the low genetic differentiation of *S. furcifera* (Sun *et al.*, 2014). Several studies have addressed the low genetic differentiation

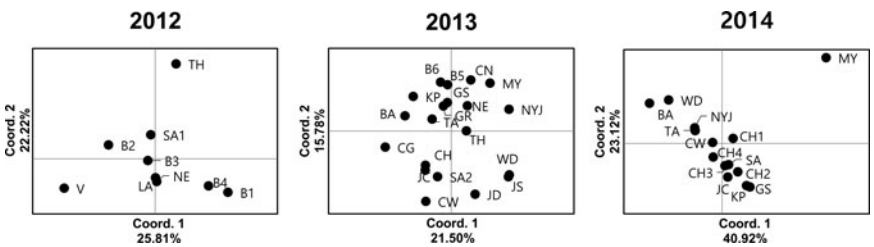

Fig. 2. Scatter diagram of factor scores from a principal coordinate analysis of genotype data for 12 microsatellite loci within samples of *S. furcifera* collected from 43 locations in 2012, 2013, and 2014. The percentage of total variation attributed to each axis is indicated.

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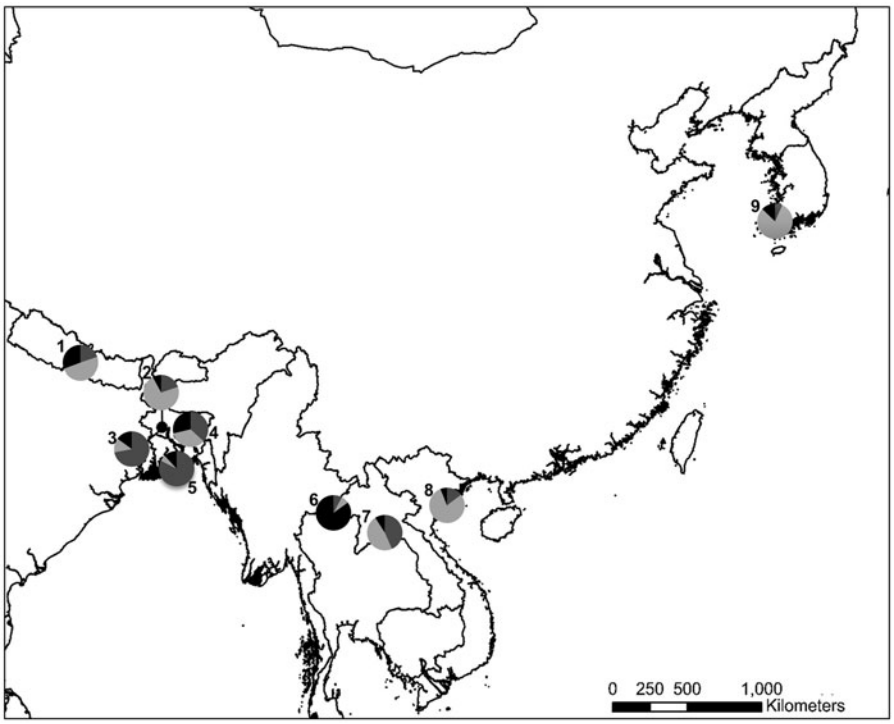

Fig. 3. All sites partitioned in three clusters ($K = 3$) and the pie graphs show the results from a Bayesian cluster analysis of multilocus microsatellite genotypes in 2012 (1 = NE, 2 = B1, 3 = B2, 4 = B3, 5 = B4, 6 = TH, 7 = LA, 8 = V, 9 = SA1).

among migratory insect populations over long distances (Malausa *et al*., 2007; Krumm *et al*., 2008). Migration route and origins of *S. furcifera* populations in Korea have been based mainly on circumstantial evidence (see Mun *et al*., 1999), but continuous efforts have been made applying molecular markers to verify the migration route and origins of *S. furcifera* populations in East Asia since Mun *et al*. (1999). Matsumoto *et al*. (2013) utilized mitochondrial COI sequence and reported no genetic structure among ten regions, supporting the hypothesis that northern Vietnam area is considered as the primary source region of *S. furcifera* to East Asia, which includes Japan, Korea, and Northern China (Sogawa, 1995;

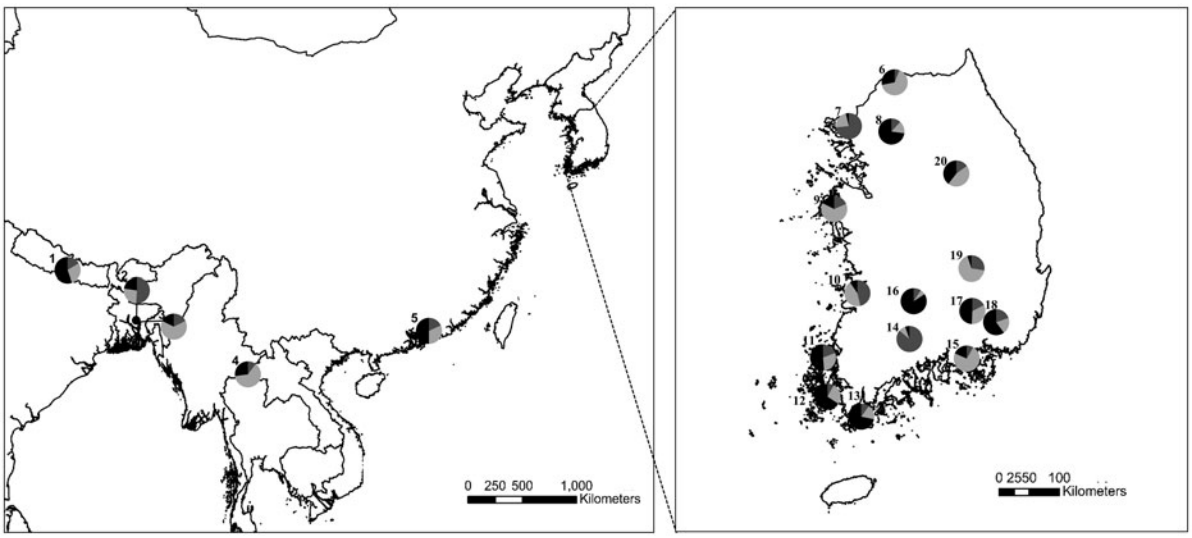

Fig. 4. All sites partitioned in three clusters ($K = 3$) and the pie graphs show the result from a Bayesian cluster analysis of multilocus microsatellite genotypes in 2013 (1 = NE, 2 = B5, 3 = B6, 4 = TH, 5 = CH, 6 = CW, 7 = KP, 8 = NYJ, 9 = TA, 10 = BA, 11 = SA2, 12 = JD, 13 = WD, 14 = GR, 15 = GS, 16 = JS, 17 = CN, 18 = MY, 19 = CG, 20 = JC).

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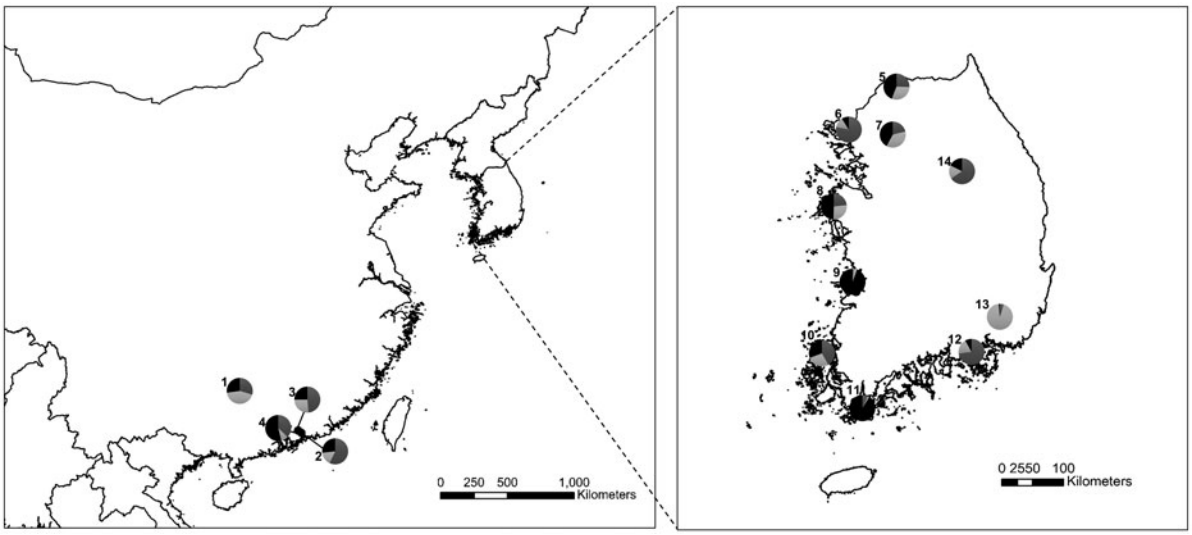

Fig. 5. All sites partitioned in three clusters ($K = 3$) and the pie graphs show the results from a Bayesian cluster analysis of multilocus microsatellite genotypes in 2014 (1 = CH1, 2 = CH2, 3 = CH3, 4 = CH4, 5 = CW, 6 = KP, 7 = NYJ, 8 = TA, 9 = BA, 10 = SA3, 11 = WD, 12 = GS, 13 = MY, 14 = JC).

Otuka *et al*., 2008). However, mitochondrial sequences did not demonstrate much differentiation of local populations and it was difficult to distinguish regional populations of *S. furcifera* based on the difference in mitochondrial sequences. In another study, Sun *et al*. (2014) applied 21 microsatellites to *S. furcifera* samples collected from five locations in China. A high degree of gene flow and genetic connectivity was evident with a low value of F_{ST}, in which five geographic populations, distanced by 414.1–1314.2 km, in China represented a single panmictic population. In contrast, Liu *et al*. (2010) showed a higher

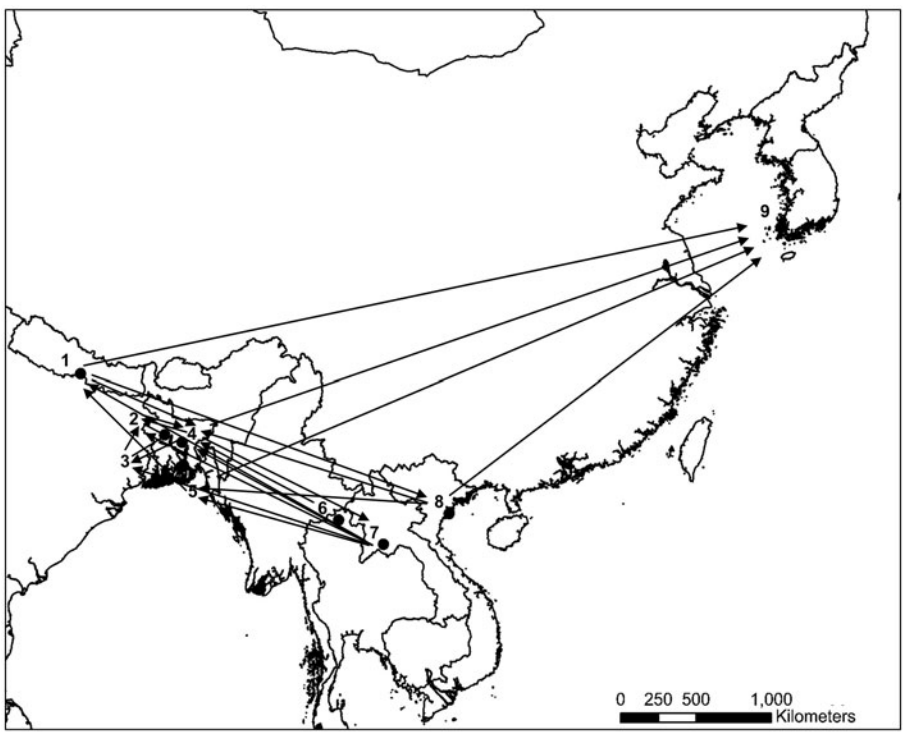

Fig. 6. Dispersal pathway of *S. furcifera* populations in Asia, which collected in 2012 (1 = NE, 2 = B1, 3 = B2, 4 = B3, 5 = B4, 6 = TH, 7 = LA, 8 = V, 9 = SA1). The arrows point to the possible source and recipient populations of first-generation immigrant detected using the L_{home}/L_{max} statistic.

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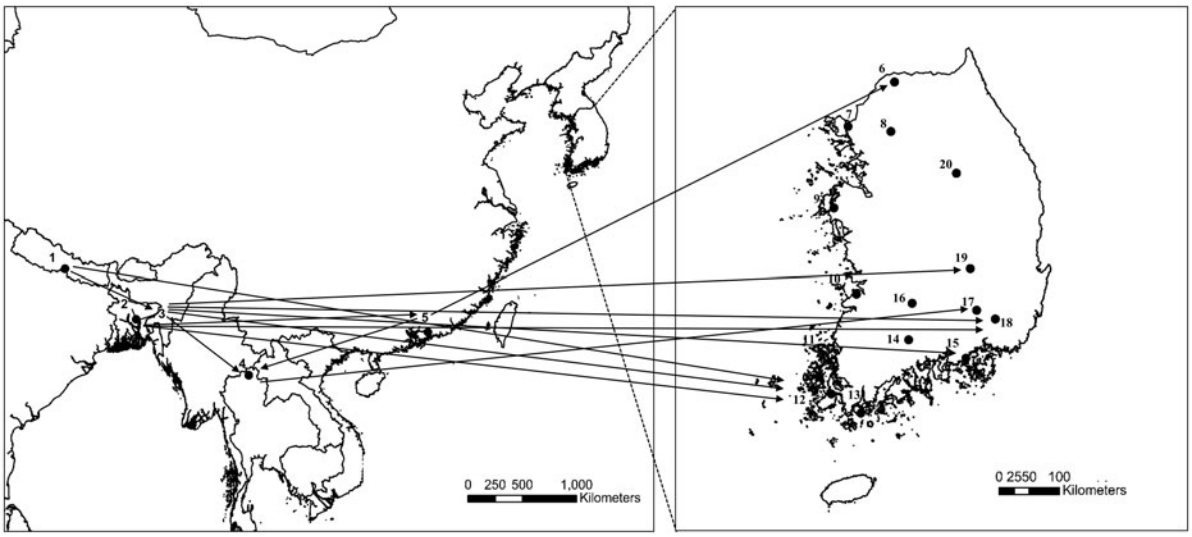

Fig. 7. Dispersal pathway of *S. furcifera* populations in Asia, which collected in 2013 (1 = NE, 2 = B5, 3 = B6, 4 = TH, 5 = CH, 6 = CW, 7 = KP, 8 = NYJ, 9 = TA, 10 = BA, 11 = SA2, 12 = JD, 13 = WD, 14 = GR, 15 = GS, 16 = JS, 17 = CN, 18 = MY, 19 = CG, 20 = JC). The arrows point to the possible source and recipient populations of first-generation immigrant detected using the L_{home}/L_{max} statistic.

level of genetic differentiation ($F_{ST} = 0.72$) among countries in Asia (11 geographic regions in Yunnan Provinces of China, Vietnam, Laos, and Myanmar) using ISSR markers. Most of the *S. furcifera* in Yunnan migrated from the adjacent southern and southwestern countries based on PCoA analysis.

In the genetic structure analysis based on the structure harvester, we revealed three genetic clusters in *S. furcifera* populations in Asia. The relationship among populations in Asia consisted of genetic connectivity and genetic divergence (figs 3–5). In the genetic cluster, there was genetic connectivity between Vietnam and Korea in 2012 and between China and Korea in 2013 and 2014. It is possible that *S. furcifera* migrates from Northern Vietnam to Korea via Southern China. In the mean assignment log-likelihood for each possible donor population in 2013, the highest value was between SA3 (Korea) and CH1 (China), and were most likely from the same source. Moreover, the genetic connection showed between TA (Korea) and CH3 (China), NYJ (Korea), and CH2 (China) in 2014, in which China might be the possible source of Korea population. Assignment testing and first-generation immigrants (L_{home}/L_{max}) revealed the possible dispersal pathway of *S. furcifera* in Asia. The analysis indicated that the source of *S. furcifera* in Korea may be China and Vietnam. However, the exact origin of the population could not be verified since

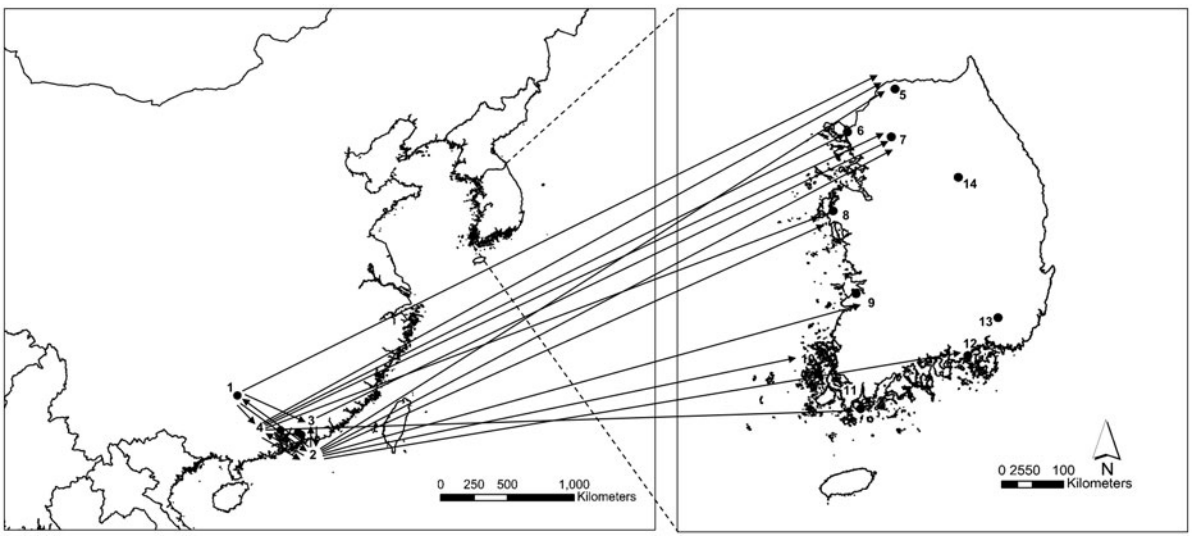

Fig. 8. Dispersal pathway of *S. furcifera* populations in Korea and China, which collected in 2014 (1 = CH1, 2 = CH2, 3 = CH3, 4 = CH4, 5 = CW, 6 = KP, 7 = NYJ, 8 = TA, 9 = BA, 10 = SA3, 11 = WD, 12 = GS, 13 = MY, 14 = JC). The arrows point to the possible source and recipient populations of first-generation immigrant detected using the L_{home}/L_{max} statistic.

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there were too few samples from various countries and there was too much gene flow among the populations. We could assume that *S. furcifera* migrates from northern Vietnam to Korea via southern China. Furthermore, the winter distribution of *S. furcifera* may be considered a key factor affecting the spatial and temporal change of population density (Hu *et al.*, 2015). Several studies indicated that *S. furcifera* overwinters in Yunnan (Liu *et al.*, 1991; Tao & Sogawa, 2000) and the temperature in Yunnan has been increasing since 1987 due to global climate change, with expansion of northern tropical and southern subtropical areas in China (Cheng *et al.*, 2014). The geographical range of *S. furcifera* overwintering population may increase in the future and migratory populations of *S. furcifera* may expand in Korea.

In conclusion, genetic diversity and connectivity among the *S. furcifera* populations in Asia based on 12 microsatellite loci indicated that, although the number of samples taken across the Asian countries in the same year was limited, there were recurrent movements of *S. furcifera* among adjacent locations. The genetic structure of *S. furcifera* populations in Korea was diverse, indicating that Korean populations have originated from diverse sources. Our results reveal that southern China and northern Vietnam are most likely the main source of *S. furcifera* populations in Korea. However, some other regions, such as Nepal and Bangladesh, might be potential sources via interconnection with Vietnam populations. Further studies are needed to examine the possibility of direct flight of *S. furcifera* from Nepal and Bangladesh to Korea. Also, future studies should examine more geographic populations in Asia collected in the same year with powerful analytical methods, such as single nucleotide polymorphism markers. These markers have higher genotyping efficiency, data quality, and level of genetic diversity compared with other molecular markers (Morin *et al.*, 2004).

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0007485318000755.

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