A THESIS FOR THE DEGREE OF

MASTER OF SCIENCE

Genetic structure of *Laodelphax striatellus* (Fallén) (Hemiptera: Delphacidae) in Korea

국내 애멸구의 유전적 구조에 대한 연구

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ABSTRACT

Genetic structure of *Laodelphax striatellus* (Fallén) (Hemiptera: Delphacidae) in Korea

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The small brown planthopper, *Laodelphax striatellus* (Fallén), is one of the serious rice pests in Asia, and transmits rice stripe virus (RSV) and rice black-streaked dwarf virus (RBSDV) to rice. *Laodelphax striatellus* overwinters in Korea and also migrates from China to western parts of Korea. Migration of *L. striatellus* has been evident since 2009.

The population genetic structure of *L. striatellus* has not been revealed in Korea. Therefore, I investigated the genetic structure of *L. striatellus* populations in spatial and temporal scales. *Laodelphax striatellus* was collected in April and September in 2013 (14 sites) and in April and July in 2014 (16 sites) in Korea. For estimating the population genetic structure of *L. striatellus*, nine microsatellite

loci were used. The average of allelic richness (A_R) ranging from 5.5 to 11.129 across populations was the lowest in the April populations in 2014. Pairwise F_{ST} values ranged from -0.0048 to 0.0484 among total genotypes. Exact tests showed no significance in all pairwise populations in April in 2013 and July in 2014. Isolation by distance (IBD) was not significant in both 2013 (r^2=0.0015, p=0.3) and 2014 (r^2=0.0041, p=0.16), indicating high gene flow among *L. striatellus* populations in Korea. Analysis of molecular variance (AMOVA) showed significantly different genetic variation among years and seasons. In principal coordinate analysis (PCoA), the April population from 2014 was separated from other groups for 21.45% for axis 1 in total genetic variance. STRUCTURE program suggested two genetic cluster, and revealed that a maximum values was 30.04 at ΔK=2, in Korea.

In addition, investigation was made to determine the ratio of wing morphs, macropter and brachypter, of *L. striatellus*. Sampling was taken at sixteen sites in April and July, 2014. Compared to the April population, ratio of brachyterous male in the July population declined significantly, while the brachypterous female ratio increased but it was not significant. A positive relationship was found between the latitude and the ratio of brachypterous adults in April.

In this study, the lack of genetic differentiation and change of proportion of the wing morph indicated the possibility of high dispersal of *L. striatellus* across the geographic areas in Korea.

Key words : microsatellite, population genetic structure, dispersal, gene flow, wing morph

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I. General introduction

Delphacidae has various serious rice pests in Asia, such as small brown planthopper (*Laodelphax striatellus* (Fallén)), brown planthopper (*Nilaparvata lugens* (Stål)), and white backed planthopper (*Sogatella furcifera* (Horvath)). *Laodelphax striatellus* causes significant damage to rice by transmitting the rice virus such as rice stripe virus (RSV) and rice black-streaked dwarf virus (RBSDV). RSV-infected rice plant shows the mosaic symptom, yellowish stripes on leaves.

Laodelphax striatellus is widely distributed in the East Asia, Russia, Northern Europe (Hyun *et al*., 1977) and rarely discovered in England (Wilson and Claridge, 1991) and Papua New Guinea (Bellis *et al*., 2014). Mass migration of *L. striatellus* from China to western parts of Korea was confirmed by Kim *et al*. (2009). The possible source of their migration appeared to be Jiangsu province in China (Otuka *et al*., 2010).

Laodelphax striatellus overwinters throughout the Korea as fourth-instar nymphs in levees mostly (Chung, 1974). Then adult *L. striatellus* begin to occur in March. They lay eggs on gramineous weed, barley and wheat, and then eggs develop to adults by mid-June, which disperse into rice fields. The 3rd and 4thgeneration adults appear in mid-July and mid-August, respectively. Also, the 5thgeneration adults occur in late September and their offspring comprise the

overwintering population. As their irregular oversea migration occur in late May-early June, nonnative and domestic populations probably exist in Korea.

The RSV occurrence was often used to interpret the change of *L. striatellus* spatial distribution indirectly. An outbreak of RSV disease was first recorded in Jinju, Miryang and Gurye in 1935. Subsequently, it spread to Chungcheongbuk-do in 1970s. The RSV-incidence has been decreased by 2000, because of widely distributed RSV-resistance variety and intensive chemical control for *L. striatellus*. After then, large trap catch of *L. striatellus* was sporadically reported in western parts in 2001, 2007 and 2009 (Kim, 2009; Otuka *et al*., 2012). Sudden increase of *L. striatellus* damages prompted to speculate increasing susceptibility of RSV-resistant rice varieties and some migration of *L. striatellus* from China. Since 2000, *L. striatellus* caused more problems in western regions than in southern regions in Korea.

Previously, molecular markers of *L. striatellus* were developed (Sun *et al*., 2012; Liu *et al*., 2013). Also, the genetic structure (Hoshizaki, 1997; Xu *et al*., 2001, Ji *et al*., 2010) and dispersal ability (Sun *et al*., 2015; Zheng *et al*., 2015) of *L. striatellus* were investigated in China and Japan. In Korea, Mun *et al*. (1999) studied genetic variation of *N. lugens* and *S. furcifera* using CO Ⅰ marker, but no population genetics study was conducted for *L. striatellus* in Korea.

Microsatellites, simple sequence repeats (SSR), are part of the junk DNA, consisting of usually di, tri, or tetra nucleotide repeats that are scattered

throughout the genome (Goldstein and Schlotterter, 1999). They are highly polymorphic, and thus can serve as codominant markers in population genetics research (Parker *et al*., 1998). The useful markers can evaluate DNA variability and differentiation among populations of insect species.

Laodelphax striatellus shows dimorphism in its wing form; macropterous (short winged morph) or brachypterous (long winged morph). The macropter is the dispersal type with flight capability for long distance movement, while the brachypter is the settlement type with high oviposition ability (Denno *et al*, 1989). The wing morph of planthoppers is developed in relation to environmental conditions, nutritious host plants, population density, temperature, habitat stability and genetic factors (Mahmud, 1980; Denno and Roderick, 1990). Hence, identifying the ratio of wing morphs is important to understand their dispersal patterns.

In this study, I used microsatellite markers for genetic structure of *L. striatellus*. The samples were collected in April and September in 2013 and April and July in 2014 in Korea. Also, I compared the ratio of wing morphs of *L. striatellus* between April and July in 2014 to elucidate how much they might disperse in Korea.

II. Materials and Methods

2-1. Sampling sites

Laodelphax striatellus was sampled at fourteen and sixteen sites in 2013 and 2014, respectively. Sampling was conducted in April and September in 2013 and April and July in 2014. The April population was composed of overwintering individuals and the July population was considered a mixture of overwintering and migration individuals. Detailed sampling information was described in Table 1 and Fig. 1.

Laodelphax striatellus was caught from forecasting plots of Agricultural Development and Technology Centers, levee and gramineous weed at each site. However in Buan, Haenam, Gurye and Miryang in April, sampling was conducted in barley or wheat fields near the forecasting plot. All samples were placed in 95.9% ethanol stored at laboratory until DNA extraction.

Table 1. Sampling information for *L. striatellus* specimens collected in 2013 and 2014 in Korea

Sample site	Sample ID	Sampling dates		coordinates
		2013	2014	
Taean	TA	15 Apr / 2 Sept	15 Apr / 22 Jul	N36° 45' 19.0", E126° 20' 40.5"
Boryeong	BR	15 Apr / 2 Sept	15 Apr / 22 Jul	N36° 23' 12.7", E126° 34' 13.8"
Buan	BA	16 Apr / 2 Sept	15 Apr / 22 Jul	N35° 44' 35.4", E126° 40' 53.9"
Shinan	SA	16 Apr / 2 Sept	15 Apr / 22 Jul	N34° 50' 44.5", E126° 21' 28.4"
Haenam	HN	16 Apr / 3 Sept	16 Apr / 23 Jul	N34° 31' 42.1", E126° 33' 34.6"
Gurye	GR	17 Apr / 5 Sept	16 Apr / 23 Jul	N35° 11' 33.5", E127° 27' 38.6"
Jinju	JJ	17 Apr / 12 Sept	16 Apr / 23 Jul	N35° 06' 50.1", E128° 10' 54.3"
Miryang	MY	17 Apr / 11 Sept	16 Apr / 24 Jul	N35° 26' 44.3", E128° 45' 25.0"
Seongju	SJ	18 Apr / 11 Sept	17 Apr / 24 Jul	N35° 54' 59.3", E128° 15' 08.3"
Yeongju	YJ	18 Apr / 10 Sept	17 Apr / 24 Jul	N36° 50' 25.1", E128° 34' 02.0"
Jecheon	JC	18 Apr / 10 Sept	17 Apr / 30 Jul	N37° 09' 38.4", E128° 10' 30.1"
Cheongju	CJ	19 Apr / 5 Sept	22 Apr / 24 Jul	N36° 35' 17.0", E127° 30' 12.8"
Chuncheon	CC	-	22 Apr / 29 Jul	N37° 56' 02.8", E127° 45' 11.5"
Gangneung	GN	-	22, 23 Apr / 29 Jul	N37° 51' 09.3", E128° 50' 38.0"
Cheorwon	CW	19 Apr / 6 Sept	22 Apr / 28 Jul	N38° 12' 05.9", E127° 15' 03.3"
Gimpo	GP	19 Apr / 6 Sept	21 Apr / 28 Jul	N37° 37' 19.9", E126° 34' 16.3"

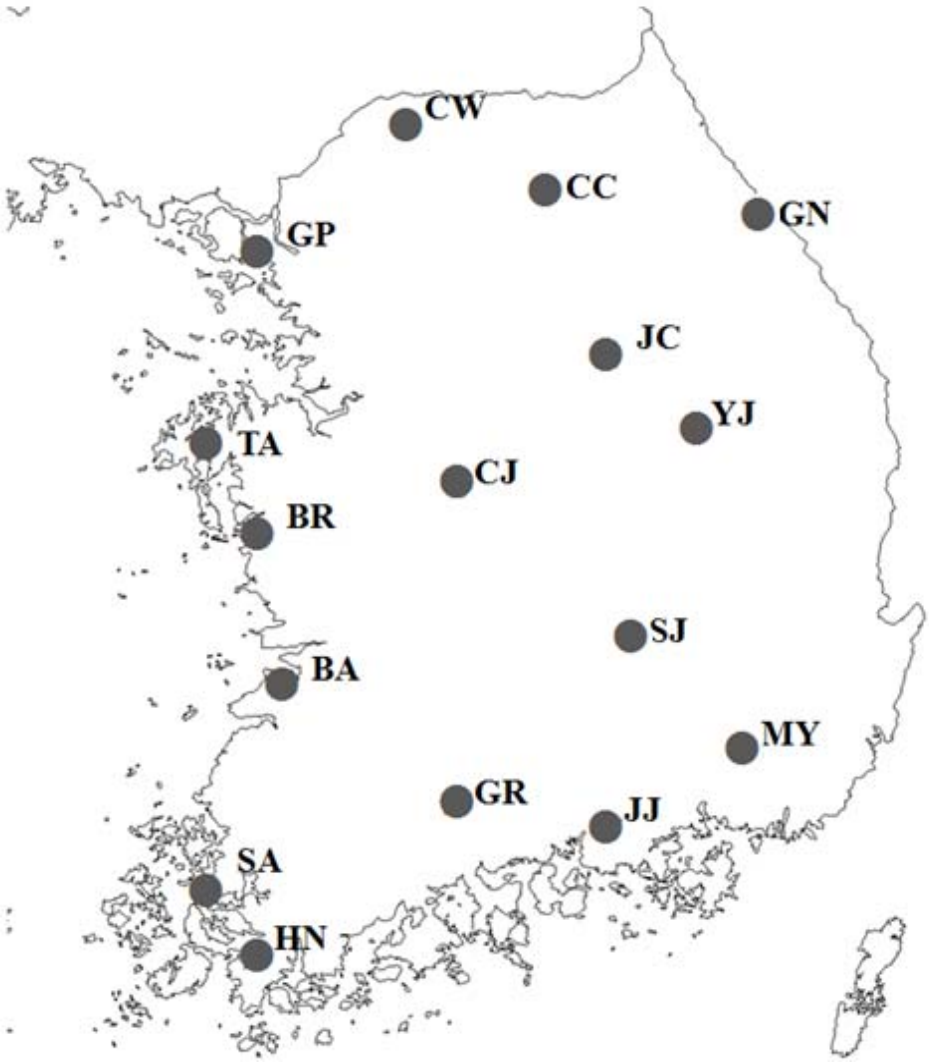

Figure 1. Map of sampling sites

2-2. Morphological and molecular identification

For morphological identification for *L. striatellus,* I observed the black face, yellowish carinae and gena (Kim *et al*., 2002). Also, morphological sex was distinguished by scutellum's color; male is black and female is brownish (Kim *et al*., 2002). Because *L. striatellus* nymphs showed a morphological variation, the molecular identification was made using COII marker (Min *et al*., 2013) (Appendix 1).

2-3. Genotyping

2-3-1. Microsatellite genotyping

DNA was extracted from *L. striatellus* individuals using Qiagen Gentra Puregen Tissue Kit (Qiagen, MD, USA). Extracted DNA were stored at -20 °C. Nine microsatellite loci previously developed for *L. striatellus* by Sun *et al*. (2012) were used. Considering the expected size range each markers, groups of multiplex polymerase chain reaction (PCR) were organized in three separate reactions; (i) for markers LS1, LS4, and LS9, (iii) for markers LS2, LS3, and LS7, and (iii) for markers LS5, LS6, and LS8. In order to analyze the length of the PCR products by a laser detection system, each of forward primer was labeled by fluorescent dye and reverse primer was unlabeled (Table 2). For these reactions, I used the rTaq PCR kit (Takara, Japan) in a total volume of 10ul, which contained 4.7ul distilled water, 1.0ul 10X PCR buffer, 1.0ul 2.5mM dNTP mixture, 0.2ul of each primer, 0.1ul of Taq polymerase, and 2.0ul template DNA. The PCR profiles followed protocol of Sun *et al*.(2012). Reactions were preceded by a 4-min denaturation step at 94 °C and were cycled 35 times with 30s at 94 °C, 30s at 55 °C, and 40s at 72 °C, followed by a final 15-min extension step at 72 °C (Sun *et al*., 2012). But observing peaks in GENEMAPPER v.3.7 (Applied Biosystems) were not clear to

calling. Thus I widely used 'Touchdown' PCR protocol (Don *et al*., 1991), whereby an initial denaturation of 4min at 95 °C was followed by five cycles of PCR, each consisting of 30s denaturation at 94 °C, 30s annealing at 65 °C, 40s extension at 72 °C and a 2 °C decrease per cycle. A total of 25cycles were then run with 15min denaturation at 72 °C. Considering analysis cost, experimental hour and allele calling efficiency multiplex PCR (Chamberlain *et al*., 1988) was conducted. Multiplex PCR products were analyzed using ABI 3730xl (Applied Biosystems). Allele size were detected using GENEMAPPER v.3.7, with ROX-500 size standard.

Table 2. Multiplex PCR information with nine primer sequence with florescent labeled dyes and GeneBank accession are shown. Nine microsatellite loci were previously developed by Sun *et al*.(2012)[1]

Multiplex group	Locus	Motif	Primer sequence (5'-3')	Size range (bp)	Dye	GeneBank Accession No.
M1	LS1	$(AC)_{5n}(AG)_6$	F: AGAGAGAGAGAGAGACACAC R: GAAAAAGCACTTGCCACATT	97-177	FAM	JN835260
	LS4	$(AC)_7$	F: TCTCTCTCTCTCTCACACAC R: GAAAATGCCAGCCGACATTC	123-157	HEX	JN835263
	LS9	$(AC)_8$	F: TCTCTCTCTCTCTCACACAC R: GAGCGAAATCCCAAAAGCA	188-262	FAM	JN835268
M2	LS2	$(AC)_5(TC)_3$	F: F: TCTCTCTCTCTCTCACACAC R: GAGGAACGAAGATAGGAAAATG	121-188	HEX	JN835261
	LS3	$(AC)_6$	F: TCTCTCTCTCTCTCACACAC R: GCGGTCGCTAATACACTCC	201-259	FAM	JN835262
	LS7	$(AC)_8$	F: AGAGAGAGAGAGAGACACAC R: CTACCATCCATCGGAATGG	91-123	FAM	JN835266
M3	LS5	$(AC)_7$	F: TCTCTCTCTCTCTCACACAC R: CGTAGGTGTCCGACTCCAAC	176-258	HEX	JN835264
	LS6	$(AC)_7$	F: AGAGAGAGAGAGAGACACAC R: TAATACAGGGTGCGTCGTTAT	126-147	FAM	JN835265
	LS8	$(AC)_{11}$	F: TCTCTCTCTCTCTCACACAC R: AACTCATTTCATAGCCCCAAC	84-142	HEX	JN835267

[1] Sun, J.T., Li, J.B., Yang, X.M., Hong X.Y., 2012. Development and characterization of nine polymorphic microsatellites for the small brown planthopper *Laodelphax striatellus* (Hemiptera: Delphacidae). Genet. Mol. Res. 11, 1526-1531

2-3-2. Statistical analysis

To calculate population genetic diversity and differentiation per locus per population, the mean number of alleles per locus, observed heterozygosity (H_O), and expected heterozygosity (H_E) under Hardy-Weinberg assumptions were estimated using the Microsatellite Toolkit (Park, 2001). The GENEPOP v.4.2 program (Raymond and Rousset, 1995) was used to test deviations from Hardy-Weinberg equilibrium (HWE) conditions. Pairwise estimates of the genetic differentiation (F_{ST}) between populations were made using FSTAT v.2.9.3 (Goudet, 2001). Micro-Checker v.2.2.3 (Oosterhout *et al*., 2004) was used to evaluate potential scoring errors, large allele drop-out, and null alleles in the *L. striatellus* microsatellite genotypes. After Micro-Checker analysis, all pairwise F_{ST} were corrected by the FreeNa program (Chapuis and Estoup, 2007) (excluding null alleles). The GenAlex v.6.5 software (Peakall and Smouse, 2006, 2012) was used carry out a principal coordinate analysis (PCoA). A scatter diagram was plotted based on factor scores along the two PCoA axis. This analysis visualizes the patterns of genetic relationship according to seasonal and different of sites. Isolation by distance (IBD) was tested by the regression of F_{ST} / (1-F_{ST}) on natural logarithm of the geographic distance between all pairs of sample sites (Rousset, 2000). IBD was used by Mantel test implemented with GenAlex v.6.5 software and result was interpreted by difference of seasonal. Analysis of molecular

variance (AMOVA) test allows the hierarchical partitioning of genetic variation among populations, sites, temporal and individuals. This analysis estimates the proportion of genetic diversity within and between populations, or among groups of populations using the random permutation approach. Hence, I calculated AMOVA for all of two years, each year and season. Genetic structure was calculated by STRUCTURE v.2.3.4 (Pritchard *et al*., 2000) that implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers.

2-4. Wing morph

2-4-1. Counting wing morph

Collected *L. striatellus* were examined using the stereoscopic microscope (Olympus SZ61, ×63) for macropterous and brachypterous wing forms, and their numbers were counted for each site, date and their sex.

2-4-2. Statistical analysis

To compare the ratio of wing morphs by site and season, a*t*-test was performed. Multiple and linear regression analyses were carried out to investigate tendency of ratio of wing morphs according to latitude, longitude and altitude. All statistical analysis was conducted using R 3.1.0 software (R Core Team, 2014). Statistical analysis was performed by converting wing morph proportion as arc sin.

III. Results

3-1. Genetic structure

3-1-1. Genetic variability

A total of 109 alleles were detected across nine microsatellite loci for 2,414 *L. striatellus* individuals from 16 sites (total 59 populations) in Korea. The number of alleles per locus ranged from 7 in CC in April in 2014 to 14 in GP in September in 2013 (mean 12.14). Genetic diversity measured for each *L. striatellus* population was deduced from the nine microsatellite loci. A_R varied from 5.137 to 11.834, and H_E ranged from 0.769 to 0.846. Total 59 populations exhibited a significant deviation from HWE following sequential Bonferroni correction for multiple testing. (Tables 3 and 4).

3-1-2. Genetic structure within and among populations

The genetic differentiation between each pair of populations for season and year is shown in Tables 5, 6, 7 and 8. Uncorrected estimates of pairwise F_{ST} values ranged from -0.0082 for the HN (April, 2014) and SJ (April, 2014) populations (ENA corrected F_{ST}=-0.0048; HN (April, 2014) and GP (April, 2014) populations) to 0.0609 for the CC (April, 2014) and GN (April, 2014) populations (ENA corrected F_{ST}=0.0484; CC (April, 2014) and GN (April, 2014) populations). Both estimates of F_{ST} were similar. Most of F_{ST} values were low and were not statistically significant. GR and TA populations in September in 2013 and GN population and eight populations (TA, BR, BA, JJ, MY, YJ, JC and CJ) in April in 2014 estimated statistically significant. However, overall, F_{ST} score range did not extend to 1 and was a low level, therefore significant *P*-values were less meaningful.

AMOVA between year, and season among the *L. striatellus* revealed that genetic variation was partitioned to among populations and individuals within populations using the random permutation approach. More than 46% of the total genetic variation was accounted for by individuals and, correspondingly, more than 37% of the total genetic variation was within individuals. But total genetic variation was ranged from 0-3% for by among year and season, year and season and 0-1% for by among populations (Table 9).

It appears that there is high gene flow among *L. striatellus* populations in Korea. Geographical distance and genetic distance among populations have no significant correlation. The Mantel tests of IBD over 2013 populations (Fig. 2a) (April, r^2=0.0085, p=0.22; September, r^2=0.0002, p=0.43) and 2014 populations (Fig. 2b) (April, r^2=0.0122, p=0.25; July, r^2=0.0246, p=0.06) demonstrate strong dispersal and high gene flow property of *L. striatellus*.

Bayesian clustering detected two clusters. The value of ΔK calculated from LnP(D) of the STRUCTURE output revealed a maximum value -80472 for K=2 among the genotypes (Table 10, Figs. 3 and 4). Overall, their genetic structure was similar between two years or seasons (Figs. 5a, b, c and d), but CC and GN on April population in 2014 (Fig. 5c) were different from others.

PCoA visualizes the pattern in genotypes of *L. striatellus* among different sites. April and September populations in 2013 were shown to be divergent by 30.98% for axis 1 (Figs. 6a, b and 7a) and also April and July populations in 2014 had divergence by 33.25% for axis 1 (Figs. 6c, d and 7b). April populations showed more separation tendency than others (Figs. 7a, b and c).

Table 3. Genetic variability estimates for each *L. striatellus* population collected in 2013, inferred from nine microsatellite loci.

Season	Sampling site	Sample size	No. of alleles	A_R	H_O	H_E	*P*-value[2]	F_{IS}	Loci with null alleles
April	TA	35	11.56	9.659	0.492	0.801	0.0002	0.39	1,2,3,4,5,6,7,8,9
	BR	36	11.67	9.598	0.473	0.778	0.0002	0.396	2,3,4,5,6,7,8,9
	BA	30	10.33	9.210	0.460	0.772	0.0002	0.409	2,4,5,6,7,8,9
	SA	36	11.33	9.478	0.433	0.790	0.0002	0.456	1,2,3,4,5,6,7,8,9
	HN	31	11.11	9.546	0.452	0.784	0.0002	0.428	2,3,4,5,6,7,8,9
	GR	43	12.56	10.066	0.508	0.800	0.0002	0.369	1,2,4,5,6,7,9
	JJ	35	11.67	9.843	0.516	0.797	0.0002	0.358	1,2,3,4,5,6,7,8,9
	MY	34	11.44	9.655	0.554	0.791	0.0002	0.304	2,3,4,5,6,7,9
	SJ	40	11.89	9.669	0.517	0.792	0.0002	0.35	2,3,4,5,6,7,8
	YJ	40	12.33	9.915	0.537	0.802	0.0002	0.334	1,2,3,4,5,6,7,9
	JC	32	11.33	9.985	0.404	0.805	0.0002	0.502	2,3,4,5,6,7,8,9
	CJ	38	12.44	10.047	0.547	0.834	0.0002	0.348	1,2,3,4,5,6,7,9
	GP	40	12.56	10.222	0.452	0.804	0.0002	0.441	2,3,4,5,6,7,8,9
September	TA	38	12.67	10.321	0.513	0.846	0.0002	0.397	1,2,3,4,5,6,7,8,9
	BR	44	13.56	10.897	0.492	0.821	0.0002	0.403	2,3,4,5,6,7,8,9
	BA	42	13.22	10.162	0.553	0.821	0.0002	0.329	1,2,3,4,5,6,7,8,9
	SA	41	12.56	10.023	0.539	0.811	0.0002	0.339	1,2,3,4,5,6,7,8,9
	HN	37	12.56	10.371	0.512	0.824	0.0002	0.383	2,3,4,5,6,7,8,9
	GR	47	12.78	9.892	0.540	0.816	0.0002	0.341	1,2,3,4,5,6,7,8
	JJ	47	13.11	10.103	0.571	0.822	0.0002	0.308	2,3,4,5,6,7,8,9
	MY	46	13.67	10.363	0.547	0.811	0.0002	0.328	1,2,3,4,5,6,7,9
	SJ	45	13.33	10.252	0.391	0.803	0.0002	0.516	1,2,3,4,5,6,7,8,9
	YJ	47	13.44	10.291	0.549	0.816	0.0002	0.33	2,3,4,5,6,7,8,9
	JC	48	13.33	9.983	0.463	0.815	0.0002	0.435	2,3,4,5,6,7,8,9
	CJ	47	13.11	10.202	0.428	0.816	0.0002	0.479	1,2,3,4,5,6,7,8,9
	CW	46	13.11	10.144	0.533	0.825	0.0002	0.356	1,2,3,4,6,7,8,9
	GP	46	13.78	10.477	0.485	0.815	0.0002	0.408	1,2,3,4,5,6,7,8,9

[2] *P*-value: Hardy-Weinberg exact test (Raymond and Rousset, 1995) with Bonferroni correction (p=0.00021)

Table 4. Genetic variability estimates for each *L. striatellus* population collected in 2014, inferred from nine microsatellite loci.

Season	Sampling site	Sample size	No. of alleles	A_R	H_O	H_E	*P*-value[3]	F_{IS}	Loci with null alleles
April	TA	46	13.11	5.772	0.358	0.820	0.0002	0.566	1,2,3,4,5,6,7,8,9
	BR	42	13.00	5.626	0.352	0.788	0.0002	0.558	1,2,3,4,5,6,7,8,9
	BA	45	11.67	5.487	0.353	0.785	0.0002	0.553	2,3,4,5,6,7,8,9
	SA	23	8.22	5.305	0.247	0.800	0.0002	0.699	1,2,3,4,5,6,7,8,9
	HN	41	12.56	5.734	0.338	0.794	0.0002	0.578	1,2,3,4,5,6,7,8,9
	GR	41	11.56	5.480	0.344	0.769	0.0002	0.556	1,2,3,4,5,6,7,8,9
	JJ	48	11.89	5.606	0.326	0.805	0.0002	0.598	2,3,4,5,6,7,8,9
	MY	48	11.78	5.409	0.308	0.773	0.0002	0.604	1,2,3,4,5,6,7,8,9
	SJ	48	12.44	5.590	0.322	0.782	0.0002	0.592	2,3,4,5,6,7,8,9
	YJ	44	11.67	5.626	0.331	0.800	0.0002	0.589	2,3,4,5,6,7,8,9
	JC	31	10.44	5.457	0.220	0.787	0.0002	0.725	2,3,4,5,6,7,8,9
	CJ	43	12.00	5.441	0.347	0.776	0.0002	0.556	1,2,3,4,5,6,7,8,9
	CC	13	7.22	5.137	0.388	0.798	0.0002	0.529	1,2,3,4,5,9
	GN	41	11.00	5.494	0.318	0.800	0.0002	0.607	1,2,3,4,5,6,7,8,9
	CW	24	9.44	5.416	0.264	0.787	0.0002	0.67	2,3,4,5,6,7,8,9
	GP	44	12.00	5.417	0.329	0.768	0.0002	0.575	1,2,3,4,5,6,7,8,9
July	TA	47	12.44	10.945	0.366	0.781	0.0002	0.534	1,2,3,4,5,6,7,8,9
	BR	44	11.44	10.225	0.347	0.771	0.0002	0.553	2,3,4,5,6,7,8,9
	BA	42	11.67	10.802	0.338	0.808	0.0002	0.585	2,3,4,5,6,7,8,9
	SA	46	12.44	10.825	0.374	0.786	0.0002	0.527	1,2,3,4,5,6,7,8,9
	HN	47	12.22	10.872	0.358	0.778	0.0002	0.544	1,2,3,4,5,6,7,8,9
	GR	46	13.22	11.532	0.434	0.809	0.0002	0.466	1,2,3,4,5,6,7,8,9
	JJ	44	12.67	11.342	0.430	0.800	0.0002	0.465	1,2,3,4,5,6,7,8,9
	MY	41	13.00	11.694	0.411	0.821	0.0002	0.502	1,2,3,4,5,6,7,8,9
	SJ	41	11.89	10.799	0.397	0.797	0.0002	0.505	2,3,4,5,6,7,8,9
	YJ	47	13.33	11.607	0.450	0.820	0.0002	0.455	2,3,4,5,6,7,8,9
	JC	43	12.56	11.195	0.403	0.799	0.0002	0.499	2,3,4,5,6,7,8,9
	CJ	39	13.33	11.834	0.421	0.802	0.0002	0.478	2,3,4,5,6,7,8,9
	CC	45	12.33	11.199	0.330	0.802	0.0002	0.592	1,2,3,4,5,6,7,8,9
	GN	42	11.89	10.924	0.318	0.803	0.0002	0.608	1,2,3,4,5,6,7,8,9
	CW	41	12.22	11.036	0.334	0.797	0.0002	0.585	1,2,3,4,5,6,7,8,9
	GP	46	12.89	11.226	0.360	0.780	0.0002	0.541	2,3,4,5,6,7,8,9

[3] *P*-value: Hardy-Weinberg exact test (Raymond and Rousset, 1995) with Bonferroni correction (p=0.00017)

Table 5. Pairwise estimates of genetic differentiation (F_{ST}) (above the diagonal) and ENA corrected F_{ST} (below the diagonal) between *L. striatellus* populations collected in April in 2013

	TA	**BR**	**BA**	**SA**	**HN**	**GR**	**JJ**	**MY**	**SJ**	**YJ**	**JC**	**CJ**	**GP**
TA	-	0.0064[4]	0.0053	0.0049	0.0096	0.0015	0.0057	0.0107	0.0052	0.0058	0.0072	0.0089	0.0023
BR	0.0067	-	0.0032	0.0067	0.0001	-0.0008	0.0046	0.0194	0.0029	0.0067	0.0035	0.0143	-0.002
BA	0.0032	0.0040	-	-0.0020	-0.0044	0.0034	0.0013	0.0129	0.0055	0.0063	0.0080	0.0094	-0.0005
SA	0.0042	0.0087	-0.0006	-	0.0003	-0.0029	-0.0017	0.0107	0.0042	0.0032	0.0088	0.0052	-0.0035
HN	0.0085	0.0029	-0.0010	0.0024	-	0.0041	0.0013	0.0144	-0.0008	0.0043	0.0034	0.0075	-0.0025
GR	0.0010	0.0024	0.0026	-0.0029	0.0047	-	-0.006	0.0069	-0.0011	-0.0027	0.0026	0.0016	-0.003
JJ	0.0037	0.0086	0.0032	0.0001	0.0047	-0.0043	-	0.0078	0.0004	0.0028	0.0054	0.0039	-0.0024
MY	0.0086	0.0203	0.0116	0.0096	0.0142	0.0060	0.0066	-	0.0115	0.0152	0.0164	0.0072	0.0140
SJ	0.0044	0.0029	0.0062	0.0020	0.0004	-0.0003	0.0009	0.0105	-	0.0035	0.0014	0.0025	0.0012
YJ	0.0042	0.0069	0.0055	0.0029	0.0045	-0.0023	0.0027	0.0123	0.0030	-	0.0074	0.0068	0.0017
JC	0.0085	0.0047	0.0103	0.0098	0.0048	0.0060	0.0097	0.0175	0.0036	0.0088	-	0.0118	-0.0014
CJ	0.0079	0.0181	0.0110	0.0055	0.0112	0.0015	0.0042	0.0085	0.0046	0.0058	0.0171	-	0.0065
GP	0.0012	0.0002	-0.0004	-0.0015	-0.0012	-0.0026	-0.0004	0.0130	0.0009	0.0012	0.0002	0.0091	-

[4] Probability of being different from zero following correction for multiple comparisons. $^{*}P<0.05$;NS, not significant. The adjusted nominal level (5%) for multiple comparisons was 0.000641. All of samples showed NS, thus omit NS.

Table 6. Pairwise estimates of genetic differentiation (F_{ST}) (above the diagonal) and ENA corrected F_{ST} (below the diagonal) between *L. striatellus* populations collected in September in 2013

	TA	**BR**	**BA**	**SA**	**HN**	**GR**	**JJ**	**MY**	**SJ**	**YJ**	**JC**	**CJ**	**CW**	**GP**
TA	-	0.0025	0.0054	0.0104	0.003	0.0159*[5]	0.0052	0.0131	0.0077	0.0130	0.0076	0.0070	0.0037	0.0048
BR	0.0060	-	0.0041	0.0039	-0.0024	0.0054	-0.0003	-0.001	-0.0006	0.0056	0.0072	0.0030	-0.0016	-0.0005
BA	0.0057	0.0054	-	-0.0043	-0.0025	0.0063	0.0033	-0.0003	-0.0012	-0.0033	0.0046	0.0004	0.0052	-0.0009
SA	0.0100	0.0059	-0.0035	-	-0.0023	0.0029	0.0014	0.0012	0.0007	-0.001	0.0028	-0.0038	0.0028	0.0011
HN	0.0020	-0.0028	-0.0017	-0.0016	-	0.0019	-0.0034	-0.0005	0.0009	-0.002	0.0012	0.0015	0.0015	0.0001
GR	0.0142*	0.0075	0.0060	0.0044	0.0019	-	0.0023	0.0056	0.0022	0.0041	0.0010	0.0061	0.0078	0.0042
JJ	0.0075	0.0018	0.0048	0.0034	-0.0027	0.0055	-	0.0034	0.0034	0.0042	0.0020	0.0050	0.0001	0.0043*
MY	0.0119	0.0017	0.0004	0.0015	-0.0018	0.0068	0.0055	-	0.0010	0.0024	0.0079	0.0038	0.0071	0.0001
SJ	0.0094	-0.0001	0.0024	0.0044	-0.0009	0.0056	0.0040	0.0036	-	0.0035	0.0029	0.0003	0.0043	-0.0049
YJ	0.0119	0.0059	-0.0025	-0.0020	-0.0016	0.0049	0.0048	0.0025	0.0049	-	0.0012	0.0013	0.0011	0.0032
JC	0.0067	0.0061	0.0047	0.0048	-0.0010	0.0023	0.0024	0.0075	0.0017	0.0021	-	0.0046	0.0048	0.0009
CJ	0.0056	0.0029	0.0019	-0.0021	-0.0007	0.006	0.0047	0.0046	0.0020	0.0013	0.0034	-	0.0062	-0.0042
CW	0.0034	0.0025	0.0035	0.0023	0.0006	0.0083	0.0022	0.0078	0.0056	0.0011	0.0038	0.0052	-	0.0077
GP	0.0052	0.0003	0.0004	0.0022	-0.0020	0.0048	0.0043*	0.0004	-0.0025	0.0029	0.0006	-0.0026	0.0072	-

[5]Probability of being different from zero following correction for multiple comparisons. *P<0.05;NS, not significant. The adjusted nominal level (5%) for multiple comparisons was 0.000549. Most of samples showed NS, thus omit NS and leave *(grey color).

Table 7. Pairwise estimates of genetic differentiation (F_{ST}) (above the diagonal) and ENA corrected F_{ST} (below the diagonal) between *L. striatellus* populations collected in April in 2014.

	TA	**BR**	**BA**	**SA**	**HN**	**GR**	**JJ**	**MY**	**SJ**	**YJ**	**JC**	**CJ**	**CC**	**GN**	**CW**	**GP**
TA	-	0.0065	0.0017	0.0066	-0.0041	0.0036	0.0008	0.0060	0.0027	-0.0039	0.0073	0.0118	0.0214	0.0204*[6]	-0.0012	0.0012
BR	0.0060	-	-0.0004	0.0165	-0.0031	0.0051	0.0038	0.0016	0.0059	0.0025	0.0040	0.0037	0.0327	0.0289*	0.0131	0.0017
BA	0.0044	0.0012	-	0.0010	-0.0041	0.0008	-0.0008	-0.0019	-0.0013	-0.0039	0.0014	-0.0020	0.0344	0.021*	0.0078	-0.0024
SA	0.0031	0.0098	0.0004	-	0.0025	0.0090	0.0007	0.0035	0.0064	0.0022	0.0099	0.0195	0.0251	0.0406	-0.0066	0.0119
HN	-0.0015	-0.0005	-0.0016	0.0009	-	-0.0043	-0.0051	0.0004	-0.0082	-0.0065	0.0024	0.0016	0.0322	0.0235	0.0008	-0.0070
GR	0.0069	0.0061	0.0027	0.0067	-0.0015	-	0.0042	0.0034	0.0017	0.0002	0.0050	0.0065	0.0354	0.0237	-0.0030	-0.0044
JJ	0.0011	0.0046	0.0002	-0.0007	-0.0031	0.0047	-	0.0014	-0.0010	-0.0080	0.0067	0.0068	0.0357	0.0252*	0.0012	-0.0001
MY	0.0073	0.0038	-0.0002	0.0031	0.0013	0.0025	0.0018	-	-0.0010	-0.0014	0.0073	0.0092	0.0462	0.0236*	0.0065	0.0025
SJ	0.0047	0.0060	-0.0008	0.0044	-0.0047	0.0026	-0.0002	0.0004	-	0.0017	0.0045	0.0092	0.0408	0.0273	0.0062	0.0001
YJ	-0.0002	0.0036	0.0007	0.0024	-0.0033	0.0004	-0.0041	0.0004	0.0032	-	0.0073	0.0047	0.0348	0.0201*	-0.0073	-0.0046
JC	0.0050	0.0039	0.0017	0.0053	0.0007	0.0055	0.0033	0.0062	0.0038	0.0050	-	0.0050	0.0268	0.0313*	0.0049	0.0047
CJ	0.0090	0.0024	0.0019	0.0105	0.0021	0.0068	0.0060	0.0071	0.0067	0.0064	0.0029	-	0.0384	0.0287*	0.0131	0.0043
CC	0.0206	0.0273	0.0356	0.0225	0.0309	0.0369	0.0327	0.0428	0.0406	0.0380	0.0295	0.0285	-	0.0609	0.0268	0.0423
GN	0.0142*	0.0201*	0.0196*	0.0244	0.0165	0.0176	0.0183*	0.0190*	0.0215	0.0147*	0.0197*	0.0199*	0.0484	-	0.0254	0.0275
CW	0.0010	0.0127	0.0065	-0.0020	0.0021	0.0010	0.0026	0.0074	0.0063	-0.0029	0.0055	0.0104	0.0316	0.0208	-	0.0007
GP	0.0023	0.0010	-0.0013	0.0071	-0.0048	-0.0021	-0.0002	0.0013	0.0004	-0.0031	0.0040	0.0039	0.0392	0.0197	0.0015	-

[6] Probability of being different from zero following correction for multiple comparisons. *P<0.05;NS, not significant. The adjusted nominal level (5%) for multiple comparisons was 0.000417. Most of samples showed NS, thus omit NS and leave *(grey color).

Table 8. Pairwise estimates of genetic differentiation (F_{ST}) (above the diagonal) and ENA corrected F_{ST} (below the diagonal) between *L. striatellus* populations collected in July in 2014

	TA	**BR**	**BA**	**SA**	**HN**	**GR**	**JJ**	**MY**	**SJ**	**YJ**	**JC**	**CJ**	**CC**	**GN**	**CW**	**GP**
TA	-	-0.0037[7]	0.0028	0.0047	0.0037	0.0043	0.0029	0.0067	0.0028	0.0051	0.0005	0.0011	0.0113	-0.0020	0.0043	-0.0017
BR	-0.0010	-	0.0044	0.0016	0.0042	0.0089	0.0008	0.0032	0.0006	0.0036	-0.0038	-0.0029	0.0022	-0.0013	0.0034	0.0016
BA	0.0040	0.0075	-	0.0030	0.0073	0.0079	0.0057	0.0057	-0.0019	0.0020	0.0019	0.0034	0.0093	-0.0015	0.0014	-0.0003
SA	0.0044	0.0064	0.0041	-	0.0005	0.0094	0.0069	0.0060	0.0066	0.0046	-0.0016	0.0019	0.0035	0.0011	0.0019	0.0066
HN	0.0034	0.0068	0.0058	0.0014	-	0.0029	0.0110	0.0059	0.0036	0.0059	-0.0034	0.0074	0.0104	0.0030	0.0057	0.0046
GR	0.0035	0.0110	0.0073	0.0100	0.0033	-	0.0054	0.0022	0.0048	0.0051	0.0009	0.0052	0.0119	0.0021	0.0043	0.0031
JJ	0.0030	0.0041	0.0037	0.0069	0.0077	0.0024	-	0.0004	0.0011	-0.0004	0.0032	-0.0006	0.0030	-0.0065	0.0003	0.0050
MY	0.0068	0.0074	0.0051	0.0069	0.0043	0.0018	-0.0007	-	-0.0029	-0.0049	-0.0057	-0.0017	-0.0002	-0.0009	0.0049	0.0067
SJ	0.0027	0.0017	0.0003	0.0078	0.0040	0.0051	0.0015	-0.0004	-	-0.0030	-0.0024	-0.0032	0.0065	0.0029	0.0029	-0.0014
YJ	0.0044	0.0045	0.0010	0.0039	0.0040	0.0044	-0.0007	-0.0029	-0.0034	-	-0.0060	-0.0018	-0.0016	0.0033	0.0013	0.0073
JC	0.0018	-0.0007	0.0033	0.0006	-0.0001	0.0040	0.0036	-0.0012	-0.0018	-0.0038	-	-0.0056	-0.0001	0.0002	0.0010	0.0028
CJ	-0.0001	-0.0005	0.0027	0.0018	0.0057	0.0046	0.0002	0.0006	-0.0017	-0.0018	-0.0031	-	0.0005	0.0005	0.0025	0.0035
CC	0.0109	0.0070	0.0072	0.0052	0.0082	0.0106	0.0030	0.0012	0.0075	-0.0003	0.0037	0.0023	-	0.0068	0.0053	0.0169
GN	0.0000	0.0040	-0.0011	0.0022	0.0022	0.0011	-0.0038	-0.0013	0.0031	0.0012	0.0019	-0.0002	0.0045	-	-0.0054	-0.0025
CW	0.0061	0.0075	0.0028	0.0026	0.0056	0.0044	0.0005	0.0029	0.0048	0.0014	0.0024	0.0005	0.0045	-0.0040	-	0.0055
GP	0.0002	0.0028	0.0037	0.0085	0.0072	0.0074	0.0074	0.0115	0.0009	0.0078	0.0025	0.0027	0.0187	0.0040	0.0092	-

[7] Probability of being different from zero following correction for multiple comparisons. $^{*}P<0.05$;NS, not significant. The adjusted nominal level (5%) for multiple comparisons was 0.000417. All of samples showed NS, thus omit NS.

Table 9. AMOVA for *L. striatellus* in Korea

Model	Source of variation	d.f.	Sums of squares	Mean sums of squares	Estimated variance	% of variation[8]	*P*-value[9]
All 59 populations From years and seasons	Between year and season	3	498.844	166.281	0.132	3%	0.001
	Among populations	55	438.624	7.975	0.026	1%	0.001
	Among individuals	2355	13697.885	5.817	2.071	53%	0.001
	Within individuals	2414	4040.000	1.674	1.674	43%	0.001
All 27 populations Between seasons in 2013	Between season	1	17.583	17.583	0.010	0%	0.001
	Among populations	25	184.577	7.383	0.023	1%	0.001
	Among individuals	1064	5872.464	5.519	1.749	46%	0.001
	Within individuals	1091	2205.000	2.021	2.021	53%	0.001
All 32 populations Between seasons in 2014	Between season	1	17.197	17.197	0.007	0%	0.001
	Among populations	30	254.047	8.468	0.029	1%	0.001
	Among individuals	1291	7825.421	6.062	2.337	62%	0.001
	Within individuals	1323	1835.000	1.387	1.387	37%	0.001
All 29 populations Between 2013 and 2014 in April	Between year	1	34.893	34.893	0.024	1%	0.001
	Among populations	27	249.085	9.225	0.045	1%	0.001
	Among individuals	1063	6220.490	5.852	2.140	57%	0.001
	Within individuals	1092	1715.500	1.571	1.571	42%	0.001
All 30 populations Between 2013 in September and 2014 in July	Between year	1	8.232	8.232	0.001	0%	0.001
	Among populations	28	189.539	6.769	0.011	0%	0.001
	Among individuals	1292	7477.395	5.787	2.015	53%	0.001
	Within individuals	1322	2324.500	1.758	1.758	46%	0.001

[8] The percentage of total variance was contributed by each component

[9] The probability test *P*-value was calculated by 999 permutations. For comparison among sites, Pennsylvania, and Oaks Corners, New York, together were considered as a single site and the remaining thirteen and fourteen sites were grouped as single site.

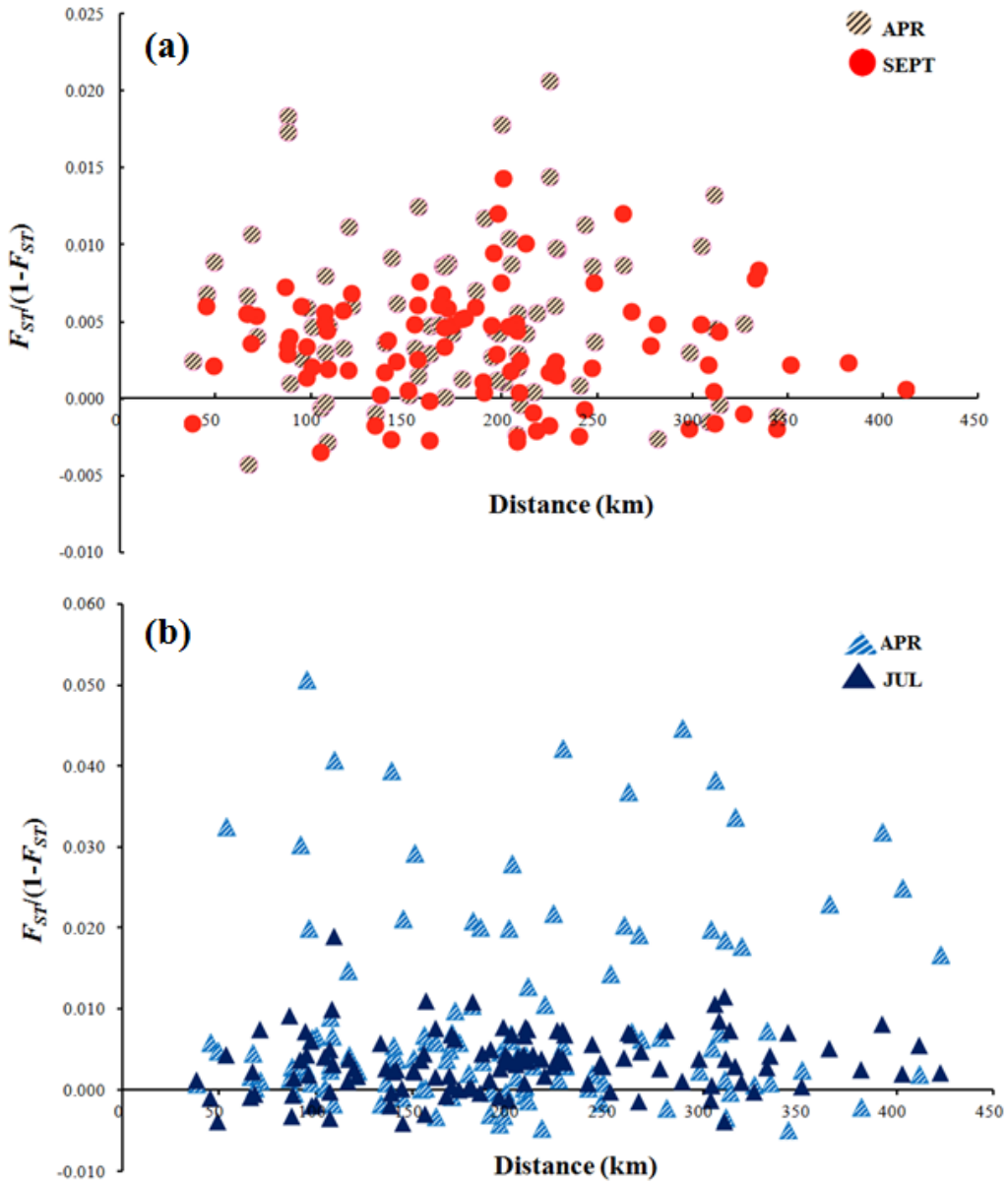

Figure 2. Geographical distance versus genetic distance (F_{ST} / 1-F_{ST}) for populations of *L. striatellus*, using pairwise F_{ST}. Correlations and probabilities were estimated from a Mantel test with 10,000 bootstrap repeats. The populations in April and September in 2013 (a) and the populations in April and July in 2014 (b). The oblique circle and triangle are in April. The dark grey circle and black triangle are in September and July, respectively.

Table 10. Likelihood values, Ln Pr(X-K), from STRUCTURE analyses (Pritchard *et al*., 2000) to determine the genetic structure of 59 populations collected in 2013 and 2014. The highest mean likelihood value (over ten runs at 400,000 replications per run) was for *K*=2 indicating the sample of individuals most likely represents two genetic population in Korea

Run	*K*=1	*K*=2	*K*=3	*K*=4	*K*=5	*K*=6	*K*=7	*K*=8	*K*=9	*K*=10
1	-80734	-80453.3	-80812.8	-81900.1	-81977.9	-83692.9	-83704.8	-85571.1	-89813.6	-96843
2	-80732.6	-80471.8	-80703.7	-82240.5	-82042.8	-82785.9	-84479.7	-86542.2	-95061.8	-94317
3	-80734.4	-80487.6	-80718.3	-81330.6	-82666	-82744.1	-84149.8	-85805	-96809.5	-93395
4	-80733.2	-80493.8	-80921.8	-82156.7	-82070	-82886.9	-84317.8	-84895.5	-95051.9	-90523
5	-80734	-80464.9	-80801.4	-81659.7	-81877.9	-84124.5	-83733.5	-94602.5	-91404.8	-90124
6	-80735.1	-80504.9	-80854.5	-82590.3	-82242	-82980.7	-83758.2	-85312.4	-95588.4	-92007
7	-80733.2	-80453	-80674.9	-81172.5	-82410.1	-83029	-84019.6	-86323.8	-94536	-91101
8	-80732.8	-80471.1	-80771.6	-81967.1	-82327.8	-83577.3	-84877.1	-91324	-95410.4	-90480
9	-80734.5	-80473.5	-80786.1	-81822.9	-82201.4	-83107.1	-85051.4	-86179.9	-91691.9	-89860.8
10	-80731.9	-80445.6	-80778.1	-82630	-81829.3	-84611.9	-84221.6	-86640	-94781.1	-86882
Mean	-80733.6	**-80472**[10]	-80782.3	-81947	-82164.5	-83354	-84231.4	-87319.6	-94014.9	-91553.3

[10] The highest mean value of Ln Pr(X-K) for each *K* is shown bold.

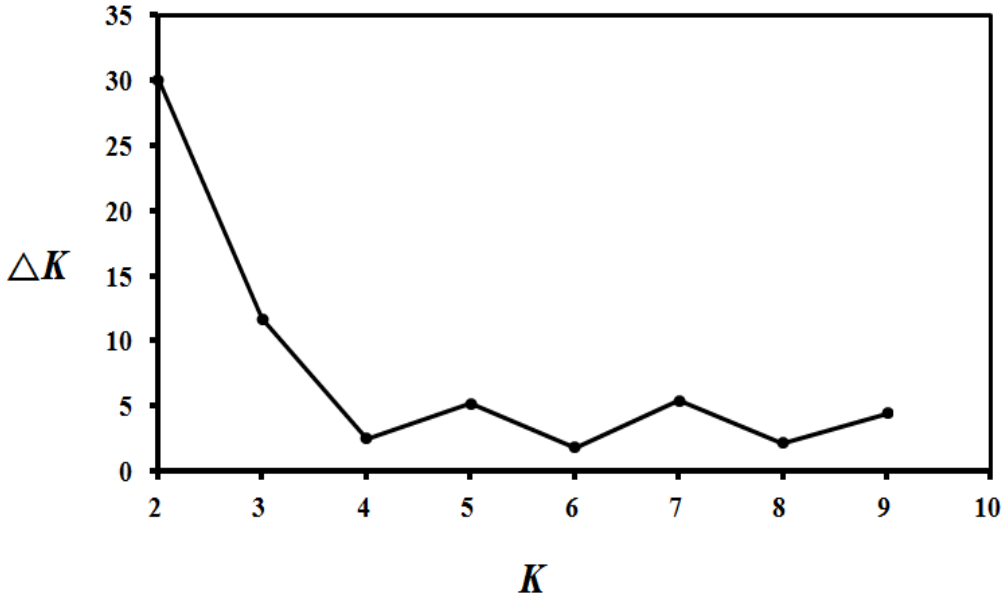

Figure 3. $\triangle K$ calculated as $\triangle K = m(|L''K|) / s[L(K)]$ (Evanno *et al*., 2005). The maximum value among genotypes was 30.04 at $\triangle K$=2.

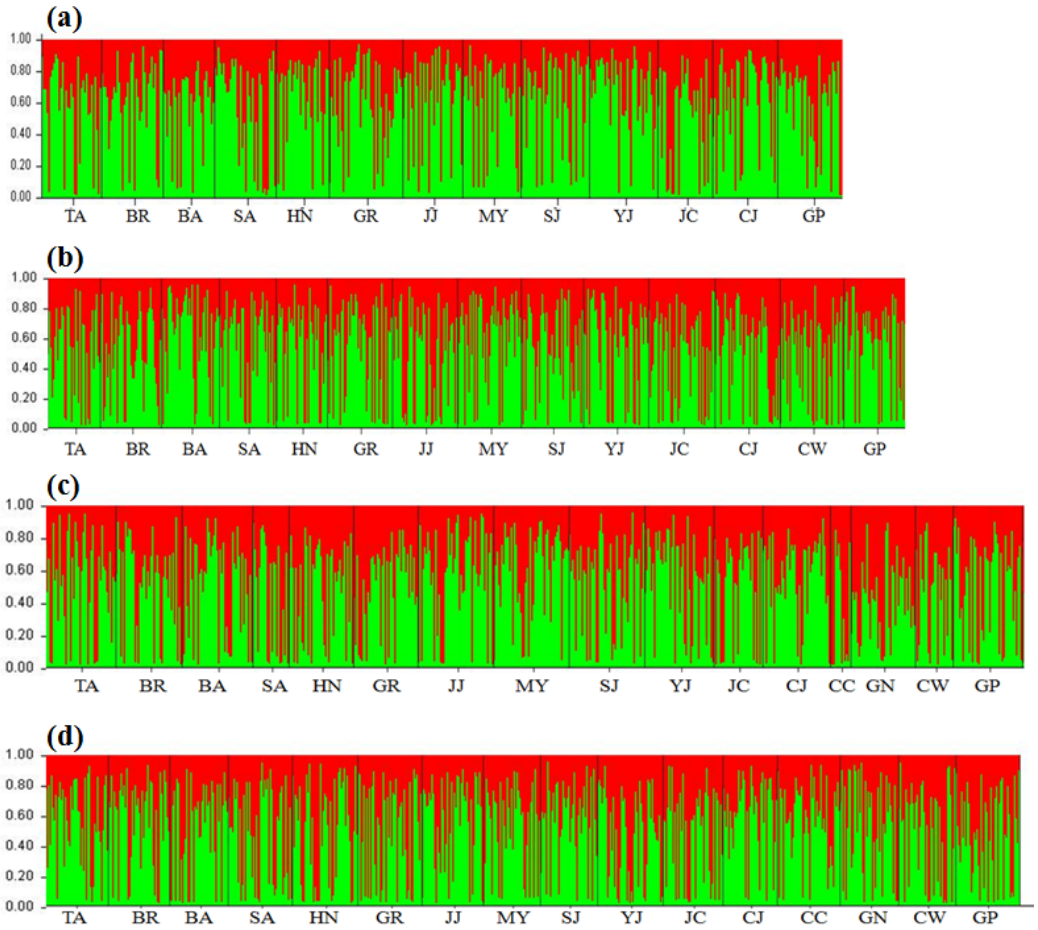

Figure 4. Bar plot of population structure estimates for 59 *L. striatellus* populations in April (a) and September (b) in 2013 and April (c) and July (d) in 2014, generated by STRUCTURE.

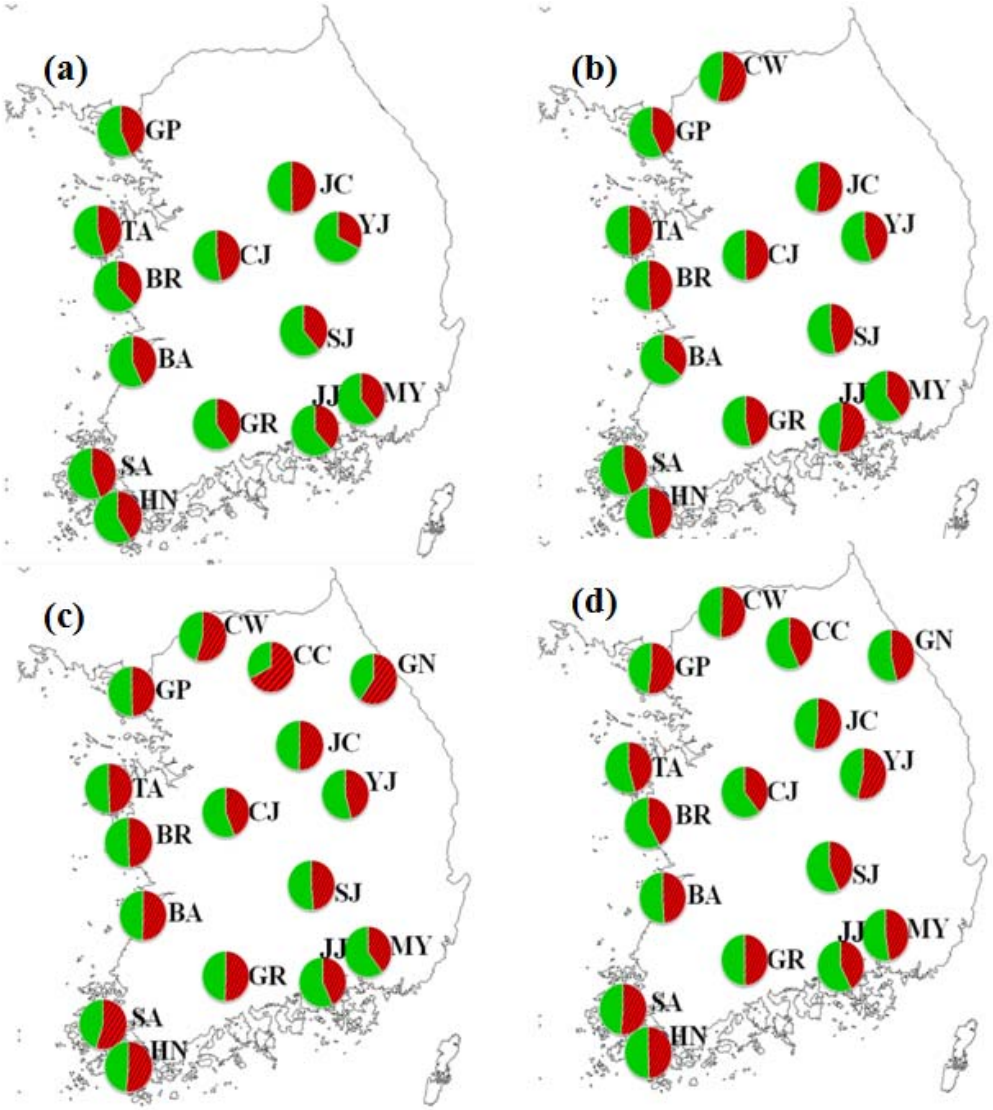

Figure 5. The pie graphs show the results of a Bayesian cluster analysis of multilocus microsatellite genotypes in April (a) and September (b) in 2013 and April (c) and July (d) in 2014. Each site is partitioned into *K*=2 components.

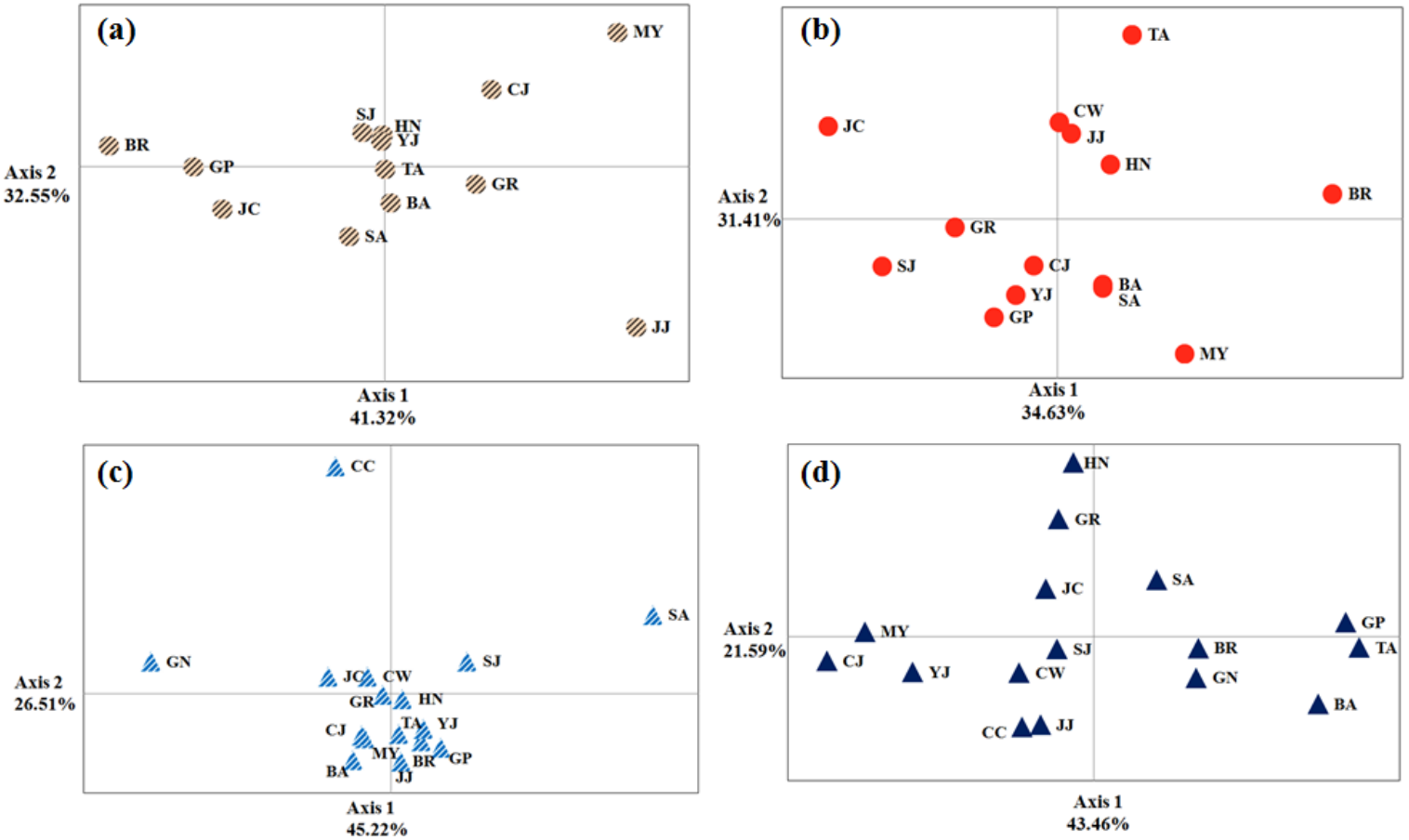

Figure 6. Scatter diagram of factor scores from a PCoA of genotype data for nine microsatellite loci in sample of *L. striatellus* collected in April (a) and September (b) in 2013 and April (c) and July (d) in 2014. The percentage of total variation attributed to each axis is indicated.

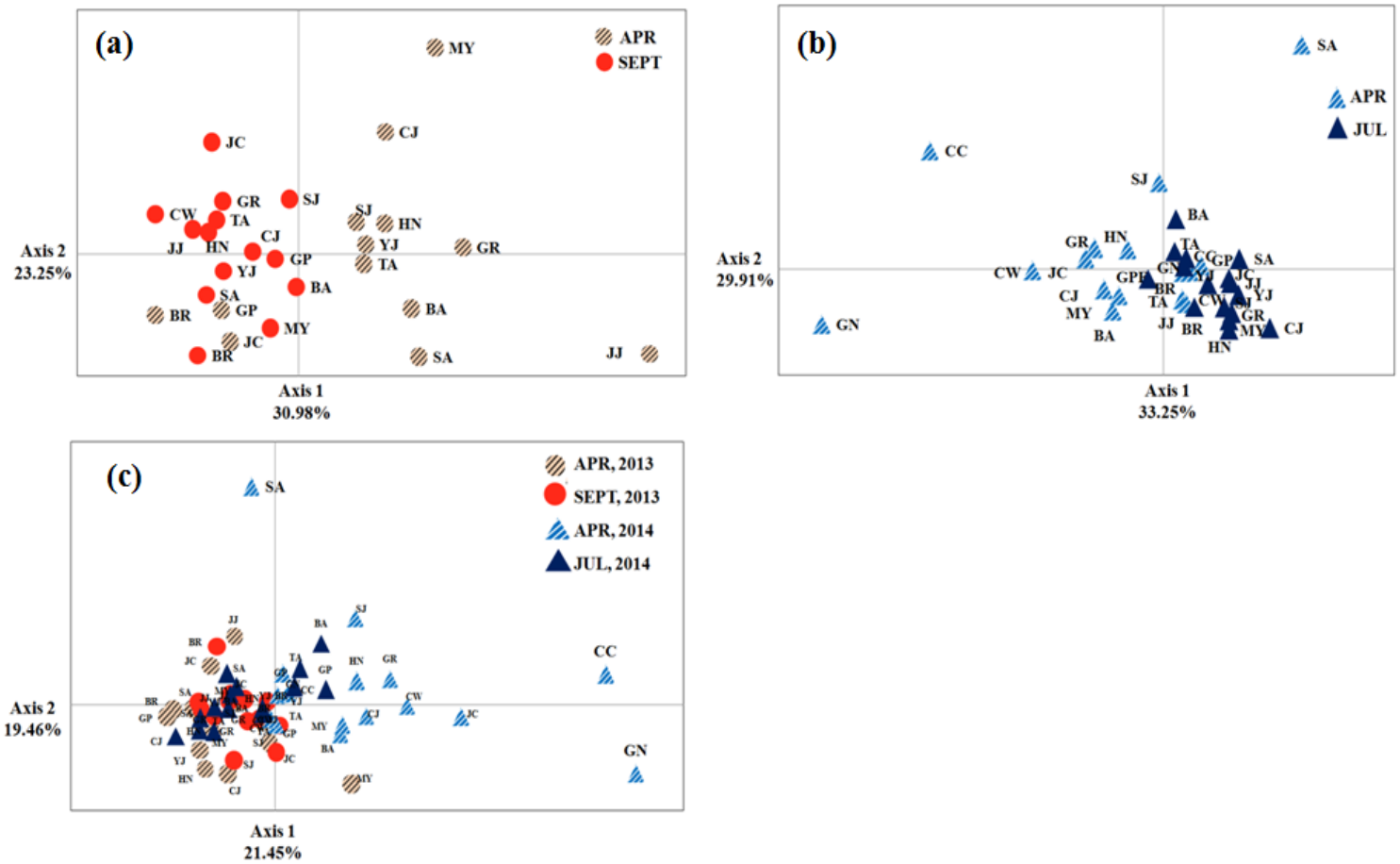

Figure 7. Scatter diagram of factor scores from a PCoA of genotype data for nine microsatellite loci in sample of *L. striatellus* collected on 2013 (a), 2014 (b) and 2013 and 2014 (c). The percentage of total variation attributed to each axis is indicated.

3-2. The patterns of wing morph

Total 5,558 individuals of *L. striatellus* were collected and examined in 2014. In April, the number of male was 770 individuals, ranging from 15 to 168 individuals per site while female was 907 individuals, ranging from 32 to 121 individuals. The number of nymphs was 222, ranging from 0 to 119 individuals. In July the number of male was 1,011, ranging from 33 to 128 individuals per site while female was 563 individuals, ranging from 2 to 117 individuals per site, and the number of nymphs was 2,085, ranging from 2 to 935 individuals (Table 11).

In April, the number of male brachypters and macropters was 157 (0-22 individuals) and 613 (11-167 individuals), respectively. The number of female brachypters and macropters was 267 (0-39 individuals) and 640 (5-121 individuals), respectively in July. Percentage of brachypterous male in July (0.3±0.2%) (mean±SE) was significantly declined compared to that in April (25.7±5.2%) (t=4.83, p<0.001), while brachypterous female in July (45.6±7.1%) increased than in April (33.9±6.8%) (t=-1.22, p=0.243) (Fig. 8a).

Percentage of wing morph was significantly changed along the latitude. The ratio of brachypters significantly increased along latitude in both male and female in April (male, y=0.1146x-3.9132, r^2=0.42; female, y=0.1446x-4.9213, r^2=0.39) (Fig. 8b).

Table11. Number of *L. striatellus* with their wing morph, sex and ratio of brachypterous individuals in each study site in 2014

Sampling site	Male				Female			
	April		July		April		July	
	No. of individuals	Ratio of brachypterous	No. of individuals	Ratio of brachypterous	No. of individuals	Ratio of brachypterous	No. of individuals	Ratio of brachypterous
GN	29	62.1%	33	0.0%	40	65.0%	80	77.5%
CW	45	48.9%	60	0.0%	46	63.0%	117	53.8%
CC	48	31.3%	57	0.0%	33	84.8%	13	7.7%
GP	60	28.3%	64	0.0%	67	20.9%	11	27.3%
MY	21	0.0%	128	0.0%	121	0.0%	14	57.1%
JJ	50	0.0%	75	0.0%	46	30.4%	17	23.5%
SJ	19	26.3%	60	0.0%	32	53.1%	34	41.2%
YJ	98	0.0%	50	2.0%	44	2.3%	13	76.9%
SA	32	15.6%	49	0.0%	45	6.7%	7	71.4%
HN	30	13.3%	119	3.4%	78	15.4%	81	59.3%
GR	168	0.6%	73	0.0%	63	0.0%	2	0.0%
BA	24	29.2%	43	0.0%	83	45.8%	66	86.4%
BR	40	47.5%	40	0.0%	59	44.1%	13	38.5%
TA	39	53.8%	40	0.0%	57	49.1%	17	23.5%
JC	52	40.4%	65	0.0%	51	54.9%	64	78.1%
CJ	15	13.3%	55	0.0%	42	7.1%	14	7.1%

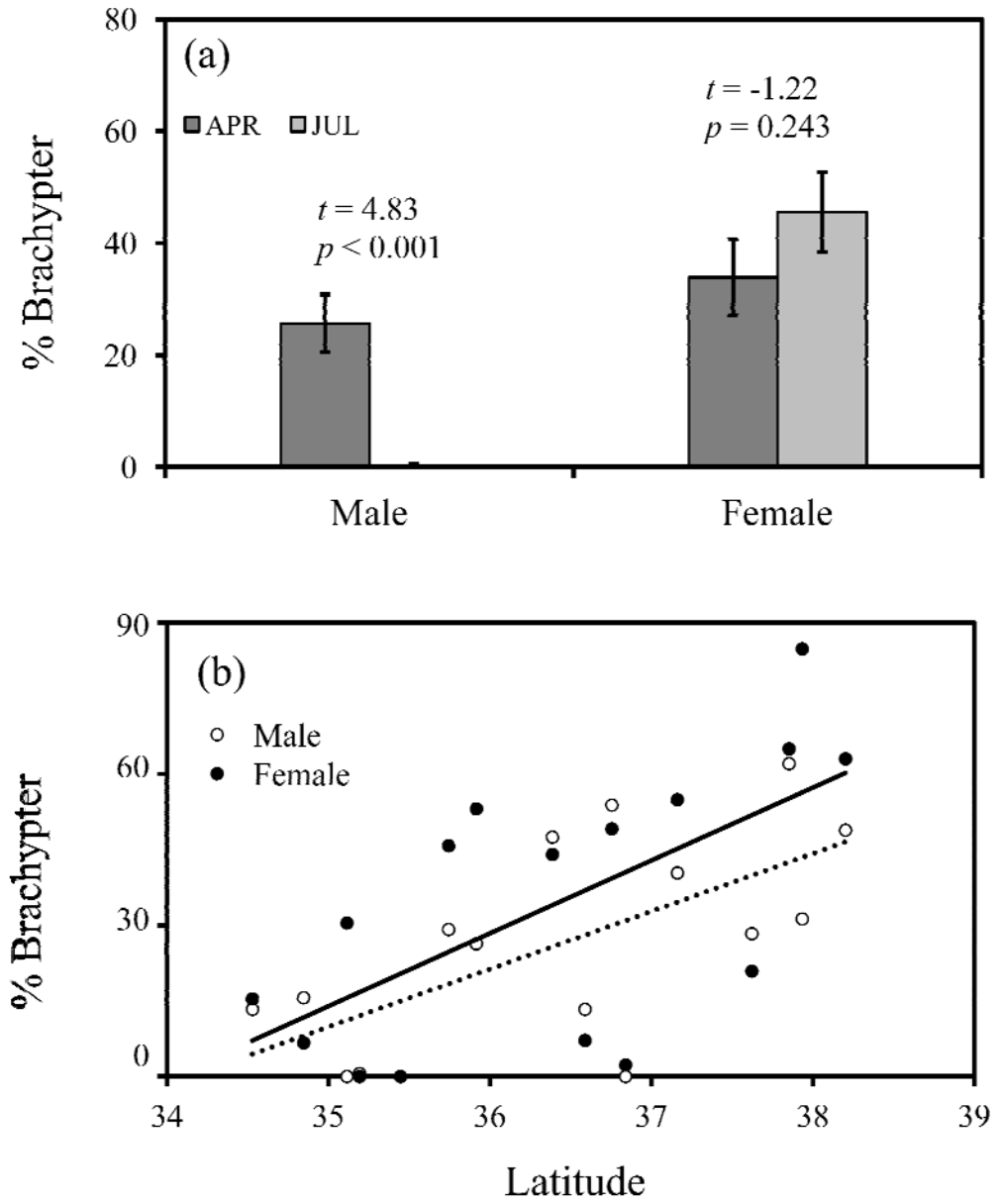

Figure 8. Comparisons of percentage of brachypterous *L. striatellus* (male and female adults) between sampling month (mean±SE) (a) and linear regressions on percentage of brachypterous male and female adults in April populations against latitude (b). Dotted- and solid-line are regression lines of male and female, respectively.

IV. Discussion

In this study, the genetic structure and gene flow of *L. striatellus* was examined using the microsatellite markers. The result indicated that, *L. striatellus* appeared to have a homogeneous genetic structure and high dispersal in Korea.

In genetics studies using microsatellite markers, frequently the high presence of null allele is inherent. Mutations in the flanking site of a microsatellite locus lead null alleles. Null alleles cause lack of binding by primers and lack of amplification of the locus. Non-amplification of one allele in a heterozygote results in only one allele being detected and false inference that the individual is a homozygote for the allele that did amplify. Although the MICRO-CHECKER program showed the probable presence of null alleles, population genetic parameters showed relatively low frequency of null allele for nine microsatellite markers ranging from 0.024 to 0.265 for *L. striatellus* populations from Jiangsu, Zhejiang and Shandong in China (Sun *et al*., 2012). Therefore, Sun *et al*. (2015) conducted genetic studies using the five microsatellite markers excluding four microsatellite markers (LS2, LS7, LS8 and LS9). In our study, the null allele frequencies ranged from -0.029 to 0.453 and 320 cases were larger than 0.2. It means the presence of high frequency of null alleles (Appendix 2). Outbreak of null alleles is frequently found in Lepidoptera, their microsatellite flanking sites present sequence similarities with a invertebrate

retrovirus and primate endogenous retrovirus, repetitive flanking sites is laboratory artifacts (Meglecz *et al*., 2004).

Sun *et al.* (2015) revealed the discordance of genetic structures *L. striatellus* between microsatellite and mtDNA in China. They speculated that this mito-nuclear discordance caused by recolonization history or mitochondria adaptation to climate. Therefore, comparing the multi-genes is needed for genetic structure studies for improving the genotypic resolution. Recently, single nucleotide polymorphisms (SNPs) for *L. striatellus* have been developed (Zheng *et al*., 2015). This is genetic polymorphism which is more sensitive and efficient, high throughput and low cost than microsatellite (Zheng *et al*., 2015). In this study, I employed only microsatellite markers and revealed no genetic differentiation in *L. striatellus* in Korea. The lack of genetic evidences prevented us from comprehending the high dispersal. Therefore, comparing the degrees of genetic differentiation including mtDNA and SNPs may help to estimate dispersal patterns of *L. striatellus* in Korea. In general, a mito-nuclear discordance caused by male-biased dispersal, asymmetric introgression of mitochondria and demographic expansion or selection on mtDNA (Pages *at al*., 2013; Toews *et al*., 2014). To identify the association with dispersal, I investigated the ratio of *L. striatellus* wing morph in Korea.

In this study, 5,558 individuals of *L. striatellus* collected in 2014 were examined for their wing morphs and the ratios of their wing morphs were

seasonally and sexually different. Overall, the proportion of brachypters was lower than that of macropters for both sexes in both April and July. Also, the proportion of brachypters was much higher in April than in July for males. In July, most of males were macropters. On the contrary, the proportion of brachypters increased in July for females. In China, the number of brachypters was significantly larger than macropters in the overwintering generation (Wang *et al*., 2013). According to Vepsäläinen (1971), the number of brachypterous *Gerris odontogaster* Zettin early summer increased. They were high reproduction more than mid July. Photoperiod during larval period was critical factor to having short wing and reproduction ability. A case of *L. striatellus*, short photoperiod in April and quality of host plant in forecasting plot may be caused to their wing morph.

The ratios of brachypterous male and female *L. striatellus* increased with the latitude. *Velarifictorus micado* decreased a rate of macropterous for increasing latitude (Zeng and Zhu, 2014). A brachypterous of *Pteremis fenestralis* (Diptera: Sphaeroceridae) was largely shown in northern sites, while macropterous was shown in southern sites in Europe (Roháček, 1975). Roháček (2012) suggested that low temperature condition restrained their flight ability. In our study, the number of brachypterous females was higher in July than in April. However, the number of brachyperous males was lower in July than in April (Fig. 8a). The rate of brachypterous females and males in April were linearly related to latitude (Fig. 8b). The cooling temperature condition of high latitude may be considered to

increasing of brachpterous of *L. striatellus* and increasing their reproduction ability.

Wing morph of male was important to mate because it associated with finding mate, host plant and habitats. Brachypterous males were more advantage of reproduction than macropterous in *Prokelisia dolus* (Langellotto *et al*., 2000). In general, flight capability and fecundity were exclusive relationship. When wings and flight muscles reduced, brachypters occurred for higher fecundity, but, when reproduction reduced, macropterous occurred for higher flight ability (Denno *et al*., 1989). The trade-offs that are flight capability and fecundity in wing morph are normal, but it could not be generalized to all species (Guerra, 2011). One sex tends to philopatric, the other have a dispersal behavior to mate (Prugnolle and de Meeus, 2002). In this view, extremely high proportion of macropterous *L. striatellus*for males in July might be their strategy of avoiding inbreeding. The philopatric of *L. striatellus* and sex-biased dispersal of male may be effected to the pattern of their wing morphs.

The very high ratio of macropters in males and sex-biased dispersal of males might cause a high gene flow and homogenous genetic structure of *L. striatellus*.

Pairwise F_{ST} values provide a measure of the genetic differentiation between populations and associated with inbreeding. The low genetic differentiation and not significant of *P*-value in all pairwise populations in our study are difficult to confirm their genetic structure. Bayesian clustering using STRUCTURE program

indicated two clusters in Korea. The total mean coefficient of ancestry for cluster 1 and cluster2 was 0.47 and 0.53, respectively. However, it was 0.60 and 0.40 in GN, and 0.67 and 0.33 in CC collected in April in 2014, respectively. Before overwintering, they may be dispersed by some factors. Giant water bug (*Lethocerus deyrollei*) migrate long distance (>3km) to find stable habitat and to select a site of overwintering (Ohba and Takagi, 2005). A wind could have caused their high gene flow and strong dispersal (Mikkola, 1986; Showers *et al*, 1995, 2001).

In this study, the result indicated a high gene flow of *L. striatellus* in Korea. Extremely high proportion (~99.7%) of macropterous male in July also indicates high dispersal and sex-biased dispersal potentials of male, resulting in a high gene flow of *L. striatellus* in Korea. However, the available microsatellites of *L. striatellus* that we used might be less sensitive to identify the genetic variations among *L. striatellus*. Study may be needed using various multigene including SNPs and mtDNA to further elucidate population genetics of *L. striatellus*.

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초 록

국내 애멸구의 유전적 구조에 대한 연구

손 병 인

애멸구 (*Laodelphax striatellus* (Fallén))는 아시아에 심각한 피해를 주는 벼 해충이며 벼줄무늬잎마름병(RSV)과 벼검은줄오갈병(RBSDV) 을 매개한다. 이 해충은 국내 대부분의 지역에서 월동 가능하며 또한 2009 년에 중국에서 국내 서해안 지역으로 비래함이 밝혀졌다.

애멸구 개체군의 유전적 구조는 국내에서 연구된 바가 없기에 본 연구에서는 국내 애멸구 개체군의 유전적 구조를 파악하고자 하였다. 연구에는 2013 년의 4 월, 9 월(14 지역)과 2014 년의 4 월, 7 월(16 지역)이라는 시간과 공간적 차이에 따라 9 개의 초위성체(Microsatellite) 마커를 이용하였다. 대립유전자형 풍부도 (A_R)의 평균은 5.5 에서 11.129 의 범위로 나타났고 2014 년 4 월에 가장 낮은 수치를 보였다. 전체 유전자형에 대한 F_{ST} 값은 −0.0048 에서 0.0484 의 범위를 보였고 P 값은 유의미하지 않았다. Isolation by distance(IBD)의 Mantel 테스트를 통해 2013 년 (r^2=0.0015, p=0.3)과 2014 년(r^2=0.0041, p=0.16)의 애멸구 개체군 간에는 유의미한 상관관계가 없었고 이는 국내에서의 높은 유전자 유동을 의미한다. Analysis of molecular variance

(AMOVA)결과, 연도와 시기 간의 유전적 변이의 유의미한 차이를 보여주었다. Principal coordinate analysis(PCoA)를 통해 2014 년의 4 월 개체군이 타 그룹에 비해 axis 1 을 기준으로 21.45%으로 분리하여 배치됨을 확인하였다. STRUCTURE 프로그램을 이용하여 유전적 클러스터를 분석한 결과, 총 2 개로 ΔK 가 2 일 때 최대값이 30.04 로 나타났다.

또한 애멸구 개체의 날개 형태(장시형과 단시형)의 비율을 조사하였다. 애멸구의 채집은 한국 16 지역에서 2014 년의 4 월과 7 월에 수행하였다. 4 월 개체군에 비해 7 월의 수컷 단시형 개체군은 급격히 감소하였고, 반면에 암컷의 비율은 증가하였다. 위도와 단시형 개체 비율의 양의 상관관계는 4 월 개체군에서 나타났다.

본 연구를 통해 국내 애멸구의 낮은 유전적 차이와 날개 형태의 비율 변화는 국내의 지리적 공간에 따른 그들의 활발한 분산 가능성을 보여주었다.

주요어 : 초위성체 마커, 개체군 유전적 구조, 분산, 유전자 유동, 날개 형태

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Appendix

Appendix 1. Characteristics of *L. striatellus* Cytochrome c oxidase subunit II (COII) loci with primer sequence, which were previously developed by Min *et al.* (2013)[11].

Locus	Sequence (5'-3')	Size range(bp)	GenBank accession No.
COⅡ_656	F: TATCTACCCGACGCATACAG R: AGATTGATTGATTCGTCCTG	516	-

[11] Min, S.J., Park, C.G., Kim, K.H., Park, H.H., Seo, B.Y., Lee, S.G., 2013. Development of species-specific primer of major Delphacidae for PCR. In: Poster competition (presentation) of 2013 Korean society of applied entomology assembly (General) meeting and spring.

Appendix 2. Frequency of null allele for each locus in the *L. striatellus* microsatellite genotypes. Frequency of null allele was estimate to Micro-Checker v.2.2.3 (Oosterhout *et al*., 2004).

Population		Frequency of null allele each locus									
		LS1	LS4	LS9	LS2	LS3	LS7	LS5	LS6	LS8	*Mean*
Apr, 2013	TA	0.177	**0.271**[12]	0.151	**0.283**	0.043	**0.236**	0.181	**0.237**	0.034	0.179
	BR	0.190	0.123	0.089	**0.230**	0.151	**0.269**	0.152	0.153	0.046	0.156
	BA	0.195	**0.239**	0.107	0.142	0.105	**0.243**	0.175	0.186	0.048	0.160
	SA	0.104	**0.203**	0.122	0.154	0.108	**0.225**	0.102	0.179	0.052	0.139
	HN	0.058	0.160	0.157	**0.247**	0.149	**0.307**	0.065	0.167	0.068	0.153
	GR	-0.002	**0.328**	**0.221**	**0.257**	**0.293**	**0.262**	**0.250**	**0.355**	0.071	0.226
	JJ	0.165	**0.313**	0.137	**0.344**	**0.240**	**0.336**	**0.281**	**0.401**	0.079	0.255
	MY	**0.294**	**0.313**	**0.226**	**0.259**	**0.306**	**0.212**	**0.323**	**0.301**	0.090	0.258
	SJ	**0.220**	0.138	0.189	0.158	0.183	**0.292**	0.179	**0.263**	0.090	0.190
	YJ	0.108	**0.284**	**0.216**	**0.248**	0.193	**0.221**	0.181	**0.308**	0.091	0.205
	JC	0.042	**0.238**	**0.263**	**0.238**	**0.350**	**0.236**	0.170	**0.389**	0.092	0.224
	CJ	0.150	**0.251**	0.069	0.193	0.185	0.187	0.144	0.182	0.093	0.161
	GP	0.064	0.143	0.188	0.198	**0.219**	**0.228**	0.163	**0.314**	0.096	0.179
Sept, 2013	TA	**0.244**	0.154	0.087	0.178	0.098	**0.306**	0.112	0.175	0.097	0.161
	BR	0.132	**0.324**	**0.228**	**0.301**	**0.286**	0.165	**0.229**	**0.389**	0.105	0.240
	BA	**0.202**	**0.278**	0.197	**0.286**	**0.214**	0.162	**0.224**	**0.312**	0.107	0.220
	SA	**0.266**	**0.324**	**0.297**	**0.298**	0.190	**0.210**	**0.359**	0.109	0.109	0.240
	HN	0.174	**0.220**	0.169	**0.231**	0.061	**0.224**	0.115	**0.203**	0.109	0.168
	GR	**0.230**	**0.245**	**0.200**	**0.331**	**0.290**	**0.261**	**0.291**	**0.327**	0.110	0.254
	JJ	0.005	0.148	0.106	**0.252**	0.148	0.167	0.066	**0.229**	0.110	0.137
	MY	0.048	**0.308**	**0.265**	**0.347**	**0.323**	**0.362**	**0.267**	**0.360**	0.110	0.266
	SJ	0.136	**0.294**	0.198	**0.309**	0.052	0.196	0.124	**0.289**	0.115	0.190
	YJ	**0.212**	0.132	0.114	0.180	0.113	**0.258**	0.163	0.181	0.117	0.163
	JC	0.196	**0.361**	0.145	**0.362**	**0.343**	**0.305**	**0.284**	**0.240**	0.123	0.262
	CJ	0.079	0.192	0.014	**0.300**	0.136	0.141	0.157	**0.298**	0.124	0.160
	CW	-0.011	**0.237**	0.134	**0.240**	**0.201**	0.087	**0.203**	**0.345**	0.124	0.173
	GP	0.085	**0.279**	**0.231**	**0.294**	**0.205**	0.170	0.188	**0.256**	0.125	0.204

[12] The values larger than 0.2 of null allele frequencies for each locus is shown in bold.

Appendix 2. Continued

Population		Frequency of null allele each locus									
		LS1	LS4	LS9	LS2	LS3	LS7	LS5	LS6	LS8	*Mean*
Apr, 2014	TA	0.128	**0.272**[13]	0.114	**0.294**	0.178	**0.204**	**0.279**	**0.284**	0.125	0.209
	BR	0.015	**0.294**	**0.223**	0.157	**0.246**	**0.273**	0.123	0.174	0.132	0.182
	BA	**0.240**	**0.400**	0.194	**0.239**	**0.338**	**0.306**	**0.337**	**0.389**	0.135	0.286
	SA	**0.203**	**0.373**	**0.256**	**0.264**	**0.262**	0.129	**0.333**	**0.320**	0.138	0.253
	HN	**0.237**	**0.291**	0.128	**0.359**	**0.344**	**0.291**	**0.201**	**0.358**	0.140	0.261
	GR	0.148	**0.349**	0.196	**0.342**	**0.238**	**0.299**	**0.257**	**0.325**	0.144	0.255
	JJ	**0.234**	**0.290**	0.154	**0.217**	0.145	0.152	0.037	0.179	0.149	0.173
	MY	0.158	**0.347**	**0.216**	**0.279**	**0.304**	**0.219**	**0.266**	**0.333**	0.150	0.253
	SJ	0.107	**0.341**	0.099	**0.328**	**0.251**	**0.347**	**0.294**	**0.319**	0.153	0.249
	YJ	0.159	**0.255**	0.167	**0.334**	**0.288**	**0.254**	**0.275**	**0.333**	0.153	0.246
	JC	**0.230**	**0.294**	**0.210**	**0.298**	0.165	0.160	0.192	**0.321**	0.154	0.225
	CJ	0.168	**0.267**	0.173	0.161	0.084	**0.319**	0.054	**0.239**	0.163	0.181
	CC	0.106	**0.317**	**0.168**	**0.343**	**0.232**	**0.281**	**0.247**	**0.352**	0.167	0.246
	GN	0.072	**0.230**	**0.204**	**0.277**	0.114	**0.212**	0.146	**0.322**	0.173	0.195
	CW	0.109	**0.334**	**0.239**	**0.389**	**0.279**	**0.324**	**0.306**	**0.296**	0.176	0.273
	GP	0.098	**0.275**	0.178	**0.234**	**0.222**	**0.226**	**0.274**	**0.324**	0.179	0.223
Jul, 2014	TA	0.192	**0.350**	0.172	**0.363**	**0.252**	**0.213**	**0.298**	**0.366**	0.198	0.267
	BR	-0.029	**0.274**	0.189	**0.248**	**0.300**	**0.221**	0.180	**0.398**	0.199	0.220
	BA	**0.203**	**0.320**	**0.200**	**0.352**	**0.296**	**0.272**	**0.229**	**0.332**	**0.200**	0.267
	SA	0.121	**0.335**	**0.245**	**0.332**	**0.300**	**0.254**	0.155	**0.337**	**0.201**	0.253
	HN	0.073	**0.250**	**0.213**	**0.247**	0.179	**0.242**	**0.229**	**0.239**	**0.202**	0.208
	GR	0.179	**0.346**	**0.221**	**0.384**	**0.236**	**0.356**	**0.341**	**0.240**	**0.203**	0.278
	JJ	0.075	**0.385**	**0.270**	**0.363**	**0.295**	**0.275**	**0.264**	**0.337**	**0.204**	0.274
	MY	**0.214**	**0.361**	**0.249**	**0.336**	**0.283**	**0.313**	**0.223**	**0.277**	**0.213**	0.274
	SJ	0.156	**0.295**	0.179	0.177	0.185	0.175	0.106	0.167	**0.217**	0.184
	YJ	**0.215**	**0.307**	**0.284**	**0.318**	**0.271**	**0.365**	**0.244**	**0.266**	**0.223**	0.277
	JC	0.065	**0.395**	**0.231**	**0.354**	**0.372**	**0.416**	**0.315**	**0.285**	**0.231**	0.296
	CJ	**0.250**	**0.330**	**0.230**	**0.320**	0.169	**0.365**	**0.206**	**0.269**	**0.241**	0.264
	CC	0.196	**0.336**	0.162	**0.345**	**0.328**	**0.259**	0.182	**0.306**	**0.251**	0.263
	GN	0.077	**0.306**	**0.201**	**0.338**	**0.325**	**0.261**	**0.283**	0.174	**0.261**	0.247
	CW	0.063	**0.378**	**0.271**	**0.360**	**0.341**	**0.419**	**0.373**	**0.393**	**0.365**	0.329
	GP	0.153	**0.453**	**0.272**	**0.251**	**0.261**	**0.334**	**0.414**	**0.288**	**0.420**	0.316

[13] The values larger than 0.2 of null allele frequencies for each locus is shown in bold.