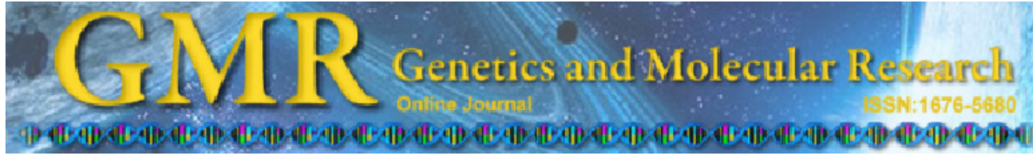

Short Communication

Development and characterization of nine polymorphic microsatellites for the small brown planthopper *Laodelphax striatellus* (Hemiptera: Delphacidae)

J.-T. Sun, J.-B. Li, X.-M. Yang and X.-Y. Hong

Department of Entomology, Nanjing Agricultural University, Nanjing, Jiangsu, China

Corresponding author: X.-Y. Hong
E-mail: xyhong@njau.edu.cn

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ABSTRACT. Nine microsatellite loci were isolated from the genome of *Laodelphax striatellus* (Homoptera: Delphacidae) by constructing $(TC)_6(AC)_5$ and $(AG)_6(AC)_5$ compound SSR-enriched libraries using suppression-PCR procedures. These loci were found to be highly polymorphic, with 13 to 30 alleles per locus in the three populations that we investigated (Jiangsu, Shandong and Zhejiang). The observed and expected heterozygosities ranged from 0.255 to 0.833 and 0.392 to 0.929, respectively. These microsatellite markers can be used for the study of population genetic structure and genetic diversity of *L. striatellus*.

Key words: SSR; Genetic structure; Genetic diversity

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INTRODUCTION

The small brown planthopper (SBPH) *Laodelphax striatellus* (Fallén) is an important agricultural pest, which is widely distributed from the Philippines to Siberia and Europe, mainly in the temperate zone (Endo et al., 2002). This pest is one of the most serious pest insects of rice plants and many other kinds of gramineous plants. In addition to the direct sucking damage, the agricultural significance of SBPH lies in its ability to spread the damaging plant disease rice stripe virus (Lijun et al., 2003).

Despite the economic importance of this pest, the population genetic structure and genetic diversity are little known. Previous studies are scarce. Using the allozyme polymorphism technique, Hoshizaki (1997) studied the geographic variation of SBPH in Japan. Xu et al. (2001) analyzed the population differentiation of three SBPH populations using the random amplified polymorphic DNA method. The molecular markers used in these two cases were not accurate enough to reveal the true patterns of genetic diversity and population structure of SBPH. In recent years, due to the characteristics of high level of polymorphism, codominant Mendelian inheritance, high frequency of occurrence, and ease of detection by polymerase chain reaction (PCR), microsatellites or simple sequence repeats have been widely used in population genetic studies, genome mapping and marker-assisted breeding (Zane et al., 2002). However, the lack of microsatellite primers developed specifically for SBPH has bottlenecked progress in the population genetic study of SBPH.

Knowledge of the genetic structure of populations of organisms provides insight into evolutionary and ecological processes. It is important to understand the genetic structure and the ecological features of local populations for effective pest management of insect populations (Hoshizaki, 1997). In this study, nine polymorphic microsatellites were first isolated from the SBPH genome using the suppression-PCR method (Lian et al., 2006). These microsatellites were tested in 141 individuals of three populations sampled from three provinces of China. High polymorphism and successful amplification rates suggest that these loci will provide powerful tools for population genetic studies in the future.

MATERIAL AND METHODS

Microsatellite development and genotyping

Microsatellites were isolated from a genomic DNA library using the suppression-PCR procedure described by Lian et al. (2006). Briefly, genomic DNA was extracted from a pool of *L. striatellus* using a CTAB method (Zhou et al., 1999). About each pool of 4 µg DNA was separately digested with blunt-end restriction enzyme, *Eco*RV, *Ssp*I, *Alu*I, *Afa*I, *Hae*III, or *Hinc*II. The restricted fragments were then ligated with a specific blunt adaptor (consisting of a 48-mer: 5'-GTAATACGATTCACTATAGGGCACGCGTGGTCGACGGCCCGGGCTGGT-3' and 8-mer with the 3'-end capped by an amino residue: 5'-ACCAGCCC-NH_2-3') by the use of T4 DNA ligase according to manufacturer instructions (Fermentas, Canada).

Fragments were amplified from DNA libraries using compound SSR primer $(TC)_6(AC)_5$ or $(AG)_6(AC)_5$ and an adaptor primer AP2 (5'-CTATAGGGCACGCGTGGT-3'). The amplified fragments were cloned into a TA cloning vector (Promega) and the plasmids were transferred to *Escherichia coli* according to manufacturer instructions. The inserted frag-

ment lengths were checked on 1.5% agarose gel electrophoresis. Amplified fragments between 300 and 800 bp were selectively sequenced. For each fragment flanking $(TC)_6(AC)_n$ or $(AG)_6(AC)_n$ compound SSR sequences at one end, a specific primer (IP1) was designed from the sequence using Primer 5.0 (http://www.premierbiosoft.com). The primer pairs of IP1 and compound SSR primer were used as a compound SSR marker.

To examine the effectiveness of primer pairs designed as a compound SSR marker, 141 *L. striatellus* adults sampled from three provinces of China (Jiangsu, Shandong and Zhejiang) were used for template DNA extraction according to protocols previously described. These populations were sampled by randomly collecting adults from 20 rice plants in an about 10 x 10-m square. PCR amplification was conducted using an Applied Biosystems Veriti™ thermal cycler (Applied Biosystems). Ten microliters of the reaction mixture contained 10-100 ng template DNA, 0.2 mM of each dNTP, 1X PCR buffer (Mg^{2+} free; Fermentas), 0.5 µM each IP1 and a dye-labeled compound SSR primer, 2.5 mM Mg^{2+}, 0.3 U Maxima Hot Start *Taq* DNA Polymerase (Fermentas). Reactions were preceded by a 4-min denaturation step at 94°C and were cycled 35 times with 30 s at 94°C, 30 s at 55°C, and 40 s at 72°C, followed by a final 15-min extension step at 72°C. PCR products were analyzed using ABI 3130 sequencer (Applied Biosystems), according to manufacturer instructions. Allele sizes were determined using GENEMAPPER version 4.0 (Applied Biosystems), with LIZ-500(-250) as size standard.

Statistical analysis

Null allele frequencies were determined with MICRO-CHECKER version 2.2.3 with the Oosterhout algorithm (Van Oosterhout et al., 2004). Expected heterozygosity (H_E), observed heterozygosity (H_O) and polymorphism information content (PIC) were calculated for each locus with Cervus version 3.0 (Marshall et al., 1998). Hardy-Weinberg equilibrium (HWE) and genotypic linkage disequilibrium between pairs of microsatellites were calculated with GENEPOP 3.4 (Raymond and Rousset, 1995). Sequential Bonferroni's correction (Rice, 1989) was applied for all multiple tests. When the hypothesis of random allele association was rejected, tests were performed using GENEPOP to find out whether deviations were the result of a deficit or an excess of heterozygotes. Pairwise F_{ST} values for each population comparison were calculated with the FSTAT2.9.3.2 software (Goudet, 1995) to determine the genetic differentiation of the three SBPH populations. We also performed a principal coordinates analysis (PCA) of the 141 individuals using the GENALEX 6 software (Peakall and Souse, 2006). Input for PCA consisted of individual pairwise genetic distance matrices of the proportion of shared alleles (Dps) calculated by the MSAnalyzer v4.05 software (Dieringer and Schlotterer, 2003).

RESULTS AND DISCUSSION

Two hundred clones were selected for sequencing. All fragments successfully sequenced flanked at least one compound microsatellite, and 100 primers were designed on 100 of the two hundred sequences. Among the 100 fragments flanking microsatellites, 62 were successfully amplified. Only 9 microsatellites (Table 1) were isolated in this study after excluding primers that amplified products that appeared to be either monomorphic or to have an unclear banding pattern in gels, and high frequency of null alleles.

Table 1. Primer sequence and characteristics of 9 microsatellite loci isolated from *Laodelphax striatellus*.

Locus	Motif	Primer sequence (5'-3')	Ta (°C)	Size range (bp)	Dye	GenBank accession No.
LS1	$(AC)_{5n}(AG)_6$	F: AGAGAGAGAGAGAGAGACACAC	55	97-177	TAMRA	JN835260
		R: GAAAAAGCACTTGCCACATT	55			
LS2	$(AC)_5(TC)_3$	F: TCTCTCTCTCTCTCACACAC	55	121-188	FAM	JN835261
		R: GAGGAACGAAGATAGGAAAATG	55			
LS3	$(AC)_6$	F: TCTCTCTCTCTCTCACACAC	55	201-259	FAM	JN835262
		R: GCGGTCGCTAATACACTCC	55			
LS4	$(AC)_7$	F: TCTCTCTCTCTCTCACACAC	55	123-157	HEX	JN835263
		R: GAAAATGCCAGCCGACATTC	55			
LS5	$(AC)_7$	F: TCTCTCTCTCTCTCACACAC	55	176-258	HEX	JN835264
		R: CGTAGGTGTCCGACTCCAAC	55			
LS6	$(AC)_7$	F: AGAGAGAGAGAGAGAGACACAC	55	126-147	FAM	JN835265
		R: TAATACAGGGTGCGTCGTTAT	55			
LS7	$(AC)_8$	F: AGAGAGAGAGAGAGAGACACAC	55	91-123	FAM	JN835266
		R: CTACCATCCATCGGAATGG	55			
LS8	$(AC)_{11}$	F: TCTCTCTCTCTCTCACACAC	55	84-142	HEX	JN835267
		R: AACTCATTTCATAGCCCCAAC	55			
LS9	$(AC)_8$	F: TCTCTCTCTCTCTCACACAC	55	188-262	TAMRA	JN835268
		R: GAGCGAAATCCCAAAAGCA	55			

Ta = annealing temperature.

The number of alleles detected in the three populations of SBPH ranged from 13 to 30 per locus, with an average of 21.44 (Table 2). The H_O and H_E across the three populations ranged from 0.298 to 0.765 and from 0.534 to 0.916, respectively. The PIC was very high and ranged from 0.534 to 0.929 (mean = 0.829). Generally, after sequential Bonferroni's correction for multiple tests, most of the loci deviated significantly from HWE due to a deficit of heterozygotes. Only six locus-population combinations were in HWE. The MICRO-CHECKER software revealed that the probable presence of null alleles for the nine loci in the three populations may contribute to the deviation of HWE. However, the null allele frequencies were relatively low. Only five cases were larger than 0.200 with the largest 0.265. All nine loci were successfully amplified and with clear band patterns in the 141 individuals. Therefore, we conclude that inbreeding may be another important factor for the deviation of HWE. The genotypic linkage disequilibrium test between pairs of microsatellites suggested that the locus LS1 has significant linkage with the LS8 locus. Pairwise estimates of F_{ST} calculated between pairs of populations indicated no significant genetic differentiation between any pairs of populations. The biggest differentiation existed between Shandong and Zhejiang (F_{ST} = 0.0022). The differentiation between Zhejiang and Jiangsu (F_{ST} = -0.0016) was similar to that between Shandong and Jiangsu (F_{ST} = -0.0006). Due to the highly migratory ability of SBPH, the resulting strong gene flow has a homogenizing effect on genetic variation over geographic populations, counteracting random drift, selection and mutation. Furthermore, PCA based on Dps also confirmed little genetic distinction between Zhejiang, Shandong and Jiangsu (Figure 1).

In conclusion, these nine new microsatellites show good polymorphism and will be powerful tools for genetic research of SBPH populations. For the three populations tested, high levels of genetic diversity and little genetic differentiation between populations were revealed by the nine microsatellites. However, only three populations were used for population genetics research. More geographic populations need to be genotyped and powerful analytical methods employed for a systematic investigation.

Table 2. Population genetic parameters for each locus and population of *Laodelphax striatellus*.

Population	Loci									Mean
	LS1	LS2	LS3	LS4	LS5	LS6	LS7	LS8	LS9	
Jiangsu										
N	48	48	48	48	48	48	48	48	48	48
N_A	14	21	20	8	19	12	12	18	20	16
H_O	0.292	0.542	0.708	0.354	0.667	0.646	0.521	0.646	0.833	0.579
H_E	0.567	0.870	0.929	0.738	0.914	0.795	0.839	0.909	0.875	0.826
Null	0.214	0.187	0.117	0.249	0.135	0.099	0.187	0.143	0.024	0.151
HWE	*	*	*	*	*	NS	*	*	NS	*
Zhejiang										
N	47	47	47	47	47	46	47	47	47	47
N_A	12	21	17	12	19	11	12	16	15	15
H_O	0.255	0.574	0.702	0.426	0.745	0.702	0.532	0.702	0.766	0.600
H_E	0.392	0.892	0.909	0.777	0.916	0.801	0.841	0.888	0.857	0.808
Null	0.144	0.178	0.112	0.220	0.094	0.057	0.186	0.106	0.051	0.127
HWE	*	*	NS	*	*	NS	*	*	NS	*
Shandong										
N	46	46	46	46	46	46	46	46	46	46
N_A	15	17	17	10	18	12	11	18	14	14.657
H_O	0.348	0.391	0.609	0.435	0.652	0.565	0.804	0.717	0.696	0.580
H_E	0.644	0.860	0.910	0.782	0.902	0.737	0.862	0.896	0.829	0.824
Null	0.211	0.265	0.163	0.211	0.138	0.109	0.031	0.098	0.081	0.145
HWE	*	*	*	*	*	*	NS	*	*	*
Mean all pops										
H_O	0.298	0.502	0.673	0.405	0.688	0.638	0.619	0.688	0.765	0.586
H_E	0.534	0.874	0.916	0.765	0.911	0.778	0.847	0.898	0.854	0.820
Total all pops										
N	141	141	141	141	141	141	141	141	141	141
N_A	26	30	24	13	25	15	14	21	25	21.444
PIC	0.534	0.884	0.929	0.779	0.923	0.786	0.854	0.910	0.863	0.829

N = number of individuals analyzed; N_A = number of alleles detected; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphism information content; Null = frequency of null allele; HWE = Hardy-Weinberg equilibrium; pops = populations. Values that are significant after sequential Bonferroni's correction for multiple tests are indicated by *P < 0.05. NS = nonsignificant.

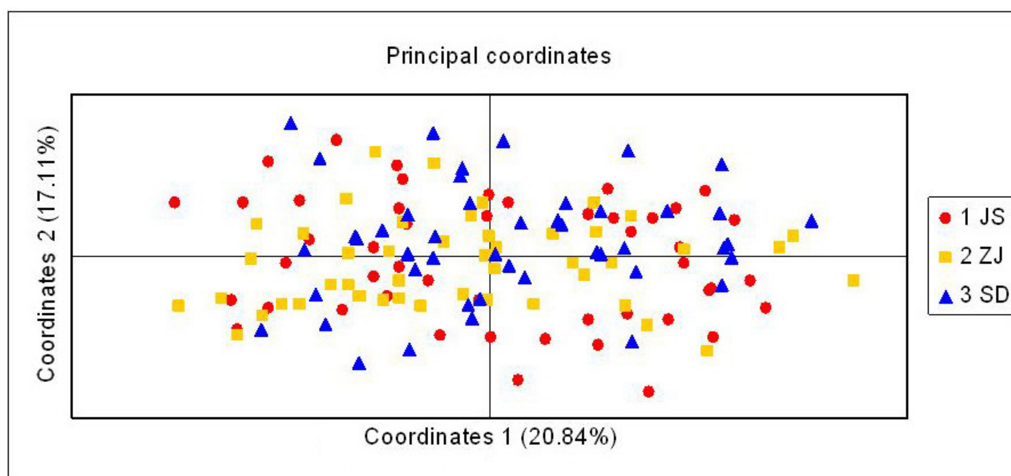

Figure 1. Principal coordinate analysis of genetic distance between 141 samples from three populations of *Laodelphax striatellus* in China. Coordinates 1 and 2 explain 37.95% of variation in the data. JS = Jiangsu; ZJ = Zhejiang; SD = Shandong.

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