



Mining and characterization of sequence tagged microsatellites from the brown planthopper *Nilaparvata lugens*

Jing-Tao Sun^a, Yan-Kai Zhang^b, Cheng Ge^c and Xiao-Yue Hong^{d*}

Department of Entomology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

Abstract

The brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is an important pest of rice. To better understand the migration pattern and population structure of the Chinese populations of *N. lugens*, we developed and characterized 12 polymorphic microsatellites from the expressed sequence tags database of *N. lugens*. The occurrence of these simple sequence repeats was assessed in three populations collected from three provinces of China. The number of alleles per locus ranged from 3 to 13 with an average of 6.5 alleles per locus. The mean observed heterozygosity of the three populations ranged from 0.051 to 0.772 and the expected heterozygosity ranged from 0.074 to 0.766. The sequences of the 12 markers were highly variable. The polymorphism information content of the 12 markers was high and ranged from 0.074 to 0.807 (mean = 0.503). Sequencing of microsatellite alleles revealed that the fragment length differences were mainly due to the variation of the repeat motif. Significant genetic differentiation was detected among the three *N. lugens* populations as the *F_{st}* ranged from 0.034 to 0.273. Principle coordinates analysis also revealed significant genetic differentiation between populations of different years. We conclude that these microsatellite markers will be a powerful tools to study the migration routine of the *N. lugens*.

Keywords: migration routine, expressed sequences, population genetics

Abbreviations: **EST**, expressed sequence tag; **GX**, Guangxi province, **JS**, Jiangsu province; **SSR**, simple sequence repeats; **ZJ**, Zhejiang province

Correspondence: ^a 2009202023@njau.edu.cn, ^b kjzhangcn@gmail.com, ^c mee@njau.edu.cn, ^d xyhong@njau.edu.cn,

* Corresponding author

Editor: Igor Sharakhov was Editor of this paper.

Received: 31 October 2010, **Accepted:** 20 May 2011

Copyright : This is an open access paper. We use the Creative Commons Attribution 3.0 license that permits unrestricted use, provided that the paper is properly attributed.

ISSN: 1536-2442 | Vol. 11, Number 134

Cite this paper as:

Sun J-T, Zhang Y-K, Ge C, Hong X-Y. 2011. Mining and characterization of sequence tagged microsatellites from the brown planthopper *Nilaparvata lugens*. *Journal of Insect Science* 11:134 available online: insectscience.org/11.134

Introduction

The brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) is an important pest of rice. It can cause hopperburn, that is characterized by complete wilting and drying of affected plants. It also transmits two rice viruses, grassy stunt and ragged stunt. *N. lugens* migrates northwards or north-eastwards from tropical and subtropical areas each year, in a series of distinct, windborne movements, progressively infesting the summer rice crop (Cheng et al. 1979). The origin of these migrations and the genetic diversity of the pest are not well known. Recent plant studies using informative genetic markers (i.e., microsatellites) have effectively revealed pollen dispersal mechanisms by analyzing the population genetic structure of reproductive trees and parentage of seedlings (Geng et al. 2008; Isagi et al. 2007). Therefore, combining sufficient microsatellite markers and direct observation methods, such as, airborne net-traps or radar, may offer additional insights into the *N. lugens* migration mechanism.

Microsatellites or simple sequence repeats (SSR) are tandemly repeated motifs of 1-6 bases found in nearly all prokaryotic and eukaryotic genomes. They are present in both coding and non-coding regions and are often characterized by a high degree of length polymorphism (Zane et al. 2002). The cause of such polymorphisms is still under debate though it appears most likely to be slippage events during DNA replication (Schlotterer et al. 1992). SSR markers have a number of characteristics that make them well suited for population genetic studies, genome mapping and marker-assisted breeding. These characteristics include a high level of polymorphism, codominant Mendelian

inheritance, a high frequency of occurrence, and ease of detection by PCR (Valdes et al. 1993; Akkaya et al. 1995; Schuler et al. 1996). Currently, only a small number of microsatellite markers have been described in the *N. lugens* (Molecular Ecology Resources Primer Development Consortium et al. 2009; Mun et al. 2004). A variety of methods for SSR isolation have been developed in recent years. The efforts required to obtain sufficient amounts of SSR primer pairs have been comprehensively reviewed by Zane et al. (2002) and Squirrell et al. (2003). However, the conventional strategies used to develop SSR markers are usually labor-intensive, time-consuming and expensive.

An enormous number of ESTs are now available in the public sequence database, and can be exploited to identify markers inexpensively. Compared with conventional markers derived from genomic DNA, EST-derived markers are easier to develop, more informative, and highly transferable. In this study, existing *N. lugens* ESTs were mined for new microsatellites to contribute to the study of *N. lugens* genetic diversity and migration routes.

Materials and Methods

Data mining

37, 348 ESTs of the *N. lugens* available as of June 2008 were downloaded from GenBank (www.ncbi.nlm.nih.gov/dbEST). The EST-trimmer software (www.pgrc.ipk-gatersleben.de/misa/download/est_trimmer.pl) was first used to remove the 5' or 3' end of polyA or polyT stretches until there were no (A)₅ or (T)₅ within the range of 50 bp, EST sequences less than 100 bp in length were discarded while only 700 bp on the 5' end were retained for ESTs greater than 700bp in

length. CAP3 software was used to assemble those sequences using the default values (Huang et al. 1999).

The obtained unigenes containing perfect SSRs were identified by the MISA software (www.pgrc.ipk-gatersleben.de/misa) and the following parameters were adopted: at least six repeats for di-, five repeats for tri-, tetra-, penta-, and hexanucleotidic. To obtain an idea about the putative functions of SSR containing genes, these sequences were compared to the nonredundant (nr) protein database of the NCBI Database (www.ncbi.nlm.nih.gov/blast) using 1e-07 as the cutoff expected value. 180 unigenes containing SSR from the unknown gene group derived from the blast were selected randomly for primer design using Primer 5.0 (www.premierbiosoft.com). The 180 unigenes selected for primer design contained only one perfect microsatellite.

***N. lugens* sampling and DNA extraction**

Three *N. lugens* populations, totaling 140 *N. lugens* adults were sampled from three provinces of China during the summer of 2008 and 2010: Guangxi Province (GX), Jiangsu Province (JS), Zhejiang Province (XJ). Information of these *N. lugens* populations is summarized in Table 1. These populations were sampled by randomly collecting adults from 20 rice plants in a 5- \times -5-m square. Genomic DNA was extracted from individual adult males while head and thorax were collected from females following the procedure of Gomi et al. (1997). Briefly, *N. lugens* individuals or head and thorax were placed in 1.5 ml microcentrifuge tubes with 25 μ l of a mixture of ice-cold STE buffer (100 mM NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 8.0) and were homogenized with a plastic pestle on ice. Proteins were removed with 2 μ l of 10 mg/ml proteinase K. The

mixtures were incubated at 37° C for 30 min, and proteinase K was inactivated at 95° C for 5 min. The DNA solution was then diluted by adding 75 μ l sterilized deionized water to each tube. The samples were briefly centrifuged and stored at -20° C for later use.

Primer screening and polymorphism detection

To examine the effectiveness of primer pairs designed to amplify SSR markers, 140 *N. lugens* adult sampled from three provinces of China were used separately for the template DNA extraction as described by Gomi et al. (1997). The forward primer of each set was tailed with AP2 [5'-CTATAGGGCACGCGTGGT-3'] to facilitate labeling. 20 μ l of the reaction mixture contained three primers (forward primer: 0.04 μ M; reverse primer: 0.2 μ M; AP2 primer: 0.2 μ M), 10-100 ng template DNA, 0.2 mmol⁻¹ of each dNTP, 1 \times PCR buffer (Fermentas, Canada, www.fermentas.com), 0.25 U of DreamTaq DNA Polymerase (Fermentas). PCR amplification was conducted on Applied Biosystems Veriti Thermal Cycler (Applied Biosystems, www.appliedbiosystems.com). The cycling conditions consisted of a touchdown regime as follows: an initial denaturing step of 3 min at 95° C, followed by 20 cycles of 30 s at 95° C, 30 s at 58° C (annealing temperature was reduced by 0.5° C per cycle), 30 s at 72° C, followed by 15 cycles of 30 s at 95° C, 30 s at 48° C, 30 s at 72° C. PCR products were analyzed using ABI 3130 sequencer (Applied Biosystems) according to the manufacturer's instructions. Allele sizes were determined using GENEMAPPER version 4.0 (Applied Biosystems), using LIZ-500(-250) as size standard.

Microsatellite sequencing

The DNA sequences of different microsatellite alleles determined by the capillary sequencer were amplified from genomic DNA by PCR in 50 μ l reactions with non-fluorescent labeling primers (conditions as above). PCR products were purified with a PCR Cleanup Kit (Axygen, www.axxygen.com) and cloned into a TA cloning vector (Invitrogen, www.invitrogen.com). The positive clones were screened and directly sequenced. Six clones were sequenced for each individual to eliminate the errors resulting from Taq polymerase misincorporation or in vitro recombinant PCR products (Ennis et al. 1990). The ClustalX (Thompson et al. 1997) program was used to compare the amplified SSR alleles.

Statistical analysis

Null allele frequencies were determined with Micro-Checker version 2.2.3 using the Oosterhout algorithm (van Oosterhout et al. 2004). Expected heterozygosity, observed heterozygosity and the polymorphism information content were calculated for each locus with Cervus version 3.0 (Marshall et al. 1998). Hardy-Weinberg equilibrium and genotypic linkage disequilibrium between pairs of microsatellites were calculated with Genepop 3.4 (Raymond et al. 1995); (Markov chain Monte Carlo parameters: 10,000 dememorization steps, 100 batches and 5000 iterations per batch). Sequential Bonferroni 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. In order to detect the genetic differentiation of the three *N. lugens* populations, pairwise F_{ST} values for each population comparison were calculated with FSTAT2.9.3.2 software (Goudet 2002). We

also performed a principal coordinates analysis of the 140 individuals using GenAlex 6 software (Peakall Souse. 2006). Input for principle coordinates analysis consisted of individual pairwise genetic distance matrices of the proportion of shared alleles calculated by MSAnalyzer v4.05 software (Dieringer and Schlotterer 2003).

Results

Data mining results

A total of 37,348 *N. lugens* ESTs were downloaded from GenBank, trimmed, and assembled into 9861 unigenes (3908 contigs and 5953 singletons) for a total length of 7.2 Mb. Of these unigenes, 465 were identified as containing SSR motifs by MISA. Of these 465 sequences, 60 had more than one SSR motif and 64 had compound SSRs. The 465 sequences included 175 (37.6%) sequences representing known genes and 290 (62.4%) that did not match any genes in GenBank. The above numbers are summarized in Figure 1.

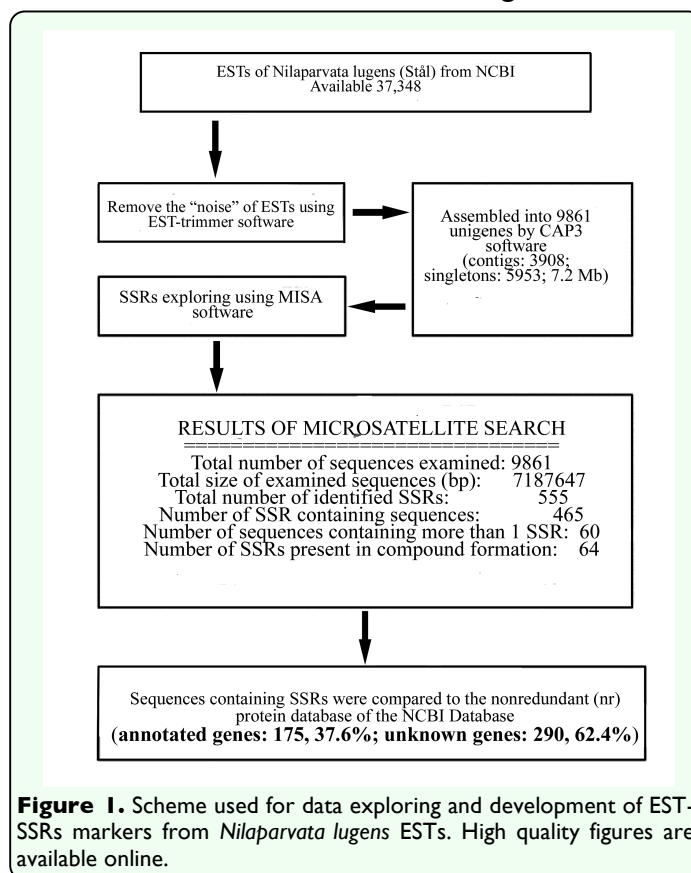


Figure 1. Scheme used for data exploring and development of EST-SSRs markers from *Nilaparvata lugens* ESTs. High quality figures are available online.

Distribution and frequencies of SSRs in *Nilaparvata lugens*

The distribution and frequencies of non-redundant EST-SSRs in the *N. lugens* transcriptome were 1 SSR/13.0 kb and most of them were smaller repeat-unit size (Table 2). Of the 555 SSR loci, 165 (29.7%) were dinucleotide repeats (DNRs). AG/CT motif was the most common among DNRs, accounting for 40.6% of the unigenes containing SSRs, followed by AT/TA (29.7%) and AC/TG (29.7%); CG/GC was not seen. The main repeat motif was trinucleotide repeats (TNRs), accounting for 65.2% of the unigenes containing SSRs. The main TNRs were AAG/TTC (30.0%) and AAT/TTA (19.1%). The frequency of other repeats motif was very low in our study, accounting for 5.0%.

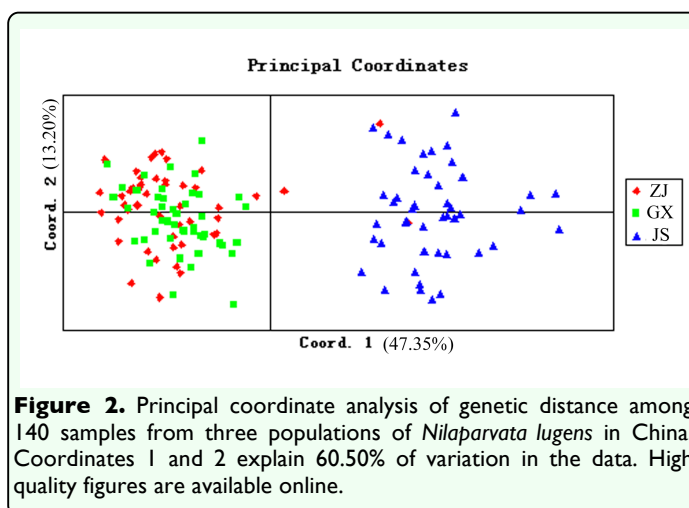
Microsatellite development and marker polymorphism

Of the 180 primer pairs, 76 primer pairs amplified the expected products, 98 primer pairs had no products and six primer sets yielded larger products indicating the existence of introns. Of the 76 successful primer pairs, 12 produced clean band patterns and revealed polymorphism among the tested brown planthopper genotypes. Primer sequence and general character of the 12 new microsatellite loci isolated in the *N. lugens* was summarized in Table 2. The number of alleles detected ranged from 3 to 13 per locus, with an average of 6.5 (Table 4). The observed and expected heterozygosity across the three populations ranged from 0.051 to 0.772 and from 0.074 to 0.764 respectively. The polymorphism information content was high and ranged from 0.074 to 0.807, with a mean of 0.503. After Sequential Bonferroni correction for multiple tests, NL177 displayed a significant departure from Hardy-Weinberg

equilibrium (HWE) due to significant heterozygote deficiency in ZJ and JS populations. In addition, the same consequence also occurred at the locus of NL22 locus in JS population. No significant heterozygote excess was detected. No linkage disequilibrium was found between any pairs of the microsatellites. Micro-Checker 2.2.3 software revealed the probable presence of null alleles for NL22, NL121, NL162, and NL177 in the three populations. However, the null allele frequencies of the four loci in three populations were very low. Only NL177 in JS population was higher than 0.200. Pairwise estimates of F_{ST} calculated between pairs of populations indicated significant genetic differentiation between populations. The biggest differentiation existed between GX and JS ($F_{ST} = 0.280$) which was similar to the differentiation between ZJ and JS ($F_{ST} = 0.273$). The GX and ZJ populations that were sampled in the same year displayed low genetic differentiation ($F_{ST} = 0.034$), but the differentiation between them was also significant ($p < 0.05$). Further principal component analysis based on genetic distance matrices of the proportion of shared alleles confirmed the genetic distinction across ZJ, GX and JS (Figure. 2).

Microsatellite mutation patterns

48 out of the total 78 alleles were sequenced successfully. The frequency of the 48 alleles



and our study. To avoid frameshift mutations in the coding regions, trinucleotide repeats motifs become the most abundant repeat motifs. Second, higher search criteria for the trinucleotide repeats motifs type SSR were used in our study (five repeats) than by Megléc et al. (2007) (four repeats). In addition, AG/TC was the predominant DNR and the CG motif was absent. Other researchers have also found the AG/CT motif to be one of the most abundant DNRs (Kantety et al. 2002; Wang et al. 2007). That may be due to the fact that a di-nucleotide motif can represent multiple codons depending on the reading frame and can translate into different amino acids. Thus the GA/CT motif can represent GAG, AGA, UCU and CUC codons in an mRNA population and translate into the amino acids Arg, Glu, Ala and Leu respectively. Ala and Leu are present in proteins at high frequencies of 8% and 10%, respectively. AAG/CTT was the most common TNR present in the *N. lugens*, and AAAT/ATTT the most common TTNR. The frequencies of TNRs and TTNRs in different organisms vary widely. The SSR frequency may help select the most suitable probe in the future when we develop SSR from the genome using traditional methods.

Characteristics of the EST-SSRs

High-resolution fingerprinting for population genetic studies requires a large number of moderately polymorphic microsatellites. Hence we tested the utility of our EST-SSRs, evaluating the polymorphism with 12 primers in a testing panel of wild *N. lugens*. Those samples were preserved in absolute ethanol and stored at -70°C for one year. DNA was extracted using a Gomi's protocol and stored for 2 months at -20°C . These DNA samples were used to select markers suitable for large-scale testing of an easy extraction method that avoids the costs and labor associated with

more elaborate extraction methods. Unigenes containing SSRs from unknown gene groups identified by the blast search were used to design primers using Primer 5.0. Thus, SSRs in these sequences may avoid selective sweeps and possess a neutral character. In addition, there are two advantages when the annealing temperature of each pair of primers is set at 55°C . First, high annealing temperatures can suppress nonspecific amplifications, which lead to unclear banding patterns. Second, the same annealing temperature is well suited for fast multiplex-PCR, which can greatly reduce labor, time and cost.

Microsatellites sequencing revealed complex mechanisms of mutations of the 12 microsatellites loci. Supporting research has also reported interspecific and intraspecific size variation at microsatellite loci that was caused solely by number variation in the primary tandem repeat unit (Angers and Bernatchez 1997; Buteler et al.1999; Colson and Goldstein 1999). For example, Matsuoka et al. (2002) reported complex mutation patterns of the maize (*Zea mays ssp. Mays*) and had minimal effects on analysis of inter- and intraspecific variation. Complex mutation of microsatellite may be a universal phenomenon in organisms. However, this research, shows insertions/indels in the regions flanking the repeat motif occurring in low frequencies. The fragment length differences were mainly a result of the variation of tandem repeat units that contained imperfect repeats and more than one type of repeat.

In spite of the complex mutation patterns, the 12 newly identified EST-SSR markers tested proved to range from moderately to highly polymorphic except the locus of NL157. In the ZJ and JS populations, two (NL22 and

NL177) of the 12 microsatellites displayed a significant departure from Hardy-Weinberg equilibrium due to significant heterozygote deficiency. Two reasons may contribute to the heterozygote deficiency. First, null alleles may be the main reason of the heterozygote deficiency. Micro-Checker software confirmed the presence of null alleles at the two loci in the population that were a departure from Hardy-Weinberg equilibrium. Another important factor was inbreeding. The *N. lugens* samples collected for this study included some brachypterous (short-winged) individuals. Unlike the macropterous form, the brachypterous form has low migration ability, and may be more likely to inbreed. From the analysis of the three *N. lugens* populations, we found that the pairwise genetic differentiation between populations sampled in different years was higher than that between populations sampled in the same year. This may be the result of different origins of *N. lugens* in different years. However, given that only three populations were analyzed in this study, developing a clearer migration path of the *N. lugens* requires analysis of more populations and more powerful analytic methods.

Acknowledgements

We thank Qi-Dong Jia, Ya-Long Xu, Guo-Qing Li, Hui Zhang, Li-Li Zhou, Ming-Zhi Yu, Xian-Ming Yang, Dong-Xiao Zhao and Ming-Hong Lu of the Department of Entomology, Nanjing Agricultural University, China for their kind help with experiments. This work was supported by grants from the National Key Basic Research Program (973 Program, No. 2006CB102001) from the Ministry of Science and Technology of China and the Science and Technology Fund of the National Public Welfare Professional Research Program (nyhyzx-200803003,

nyhyzx-200903051) from the Ministry of Agriculture of China, and a Grant-in-Aid for Scientific Research (No. 30871635) from the National Natural Science Foundation of China.

References

- Akkaya MS, Shoemaker RC, Specht JE, Bhagwat AA, Cregan PB. 1995. Integration of simple sequence repeat and markers into a soybean linkage map. *Crop Science* 35: 1439-1445.
- Angers B, Bernatchez L. 1997. Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. *Molecular Biology Evolution* 14: 230-238.
- Buteler MI, Jarret RL, LaBonte DR. 1999. Sequence characterization of microsatellites in diploid and polyploid *Ipomoea*. *Theoretical and Applied Genetics* 99: 123-132.
- Cheng SN, Chen JC, Si H, Yan LM, Chu TL, Wu CT, Chien JK, Yan CS. 1979. Studies on the migration of brown planthopper *Nilaparvata lugens* (Stål). *Acta Entomologica Sinica* 22: 1-21.
- Colson I, Goldstein DB. 1999. Evidence for complex mutations at microsatellite loci in *Drosophila*. *Genetics* 152: 617-627.
- Dieringer D, Schlotterer C. 2003. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes* 3:167-169.
- Ennis PD, Zemmour J, Salter RD, Parham P. 1990. Rapid cloning of HLA-A, B cDNA by using polymerase chain reaction: frequency

and nature of errors produced in amplification. *Proceedings of the National Academy of Sciences USA* 87: 2833-2837.

Geng Q, Lian C, Goto S, Tao J, Kimura M, Islam MD, Hogetsu T. 2008. Mating system, pollen and propagule dispersal, and spatial genetic structure in a high-density population of the mangrove tree *Kandlia candel*. *Molecular Ecology* 17: 4724-4739.

Gomi K, Gotoh T, Noda H. 1997. *Wolbachia* having no effect on reproductive incompatibility in *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). *Applied Entomology and Zoology* 32: 485-490.

Goudet J. 2002. FSTAT, a program to calculate F-Statistics. *Journal of Heredity* 86: 485-486.

Huang X, Madan A. 1999. CAP3: a DIVA sequence assembly program. *Genome Research* 9: 868-877.

Isagi Y, Saito D, Uchi HK, Tateno RS, Watanabe S. 2007. Effective pollen dispersal in enhanced by the genetic structure of an *Aesculus turbinata* population. *Journal of Ecology* 95: 983-990.

Kantety RM, Matthews DE, Sorrells ME. 2002. Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. *Plant Molecular Biology* 148: 501-510.

Marshall TC, Slate J, Kruuk L, Pemberton JM. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7: 639-655.

Matsuoka Y, Mitchell SE, Kresovich S, Goodman M, Doebley J. 2002. Microsatellites in *Zea*-variability, patterns of mutations, and

use for evolutionary studies. *Theoretical and Applied Genetics* 104: 436-450.

Megléc E, Anderson SJ, Bourguet D, Butcher R, Caldas A, Cassel-Lundhagen A, d'Acier AC, Dawson DA, Faure N, Fauvelot C, Franck P, Harper G, Keyghobadi N, Kluetsch C, Muthulakshmi M, Nagaraju J, Patt A, Péténian F, Silvain JF, Wilcock HR. 2007. Microsatellite flanking region similarities among different loci within insect species. *Insect Molecular Biology* 16: 175-185.

Molecular Ecology Resources Primer Development Consortium, Abercrombie, L.G., Anderson, C.M. Baldwin BG, Bang IC, Beldade R, Bernard G, Boubou A, Branca A, Bretagnolle F, Bruford MW, Buonamici A, Burnett Jr RK, Canal D, Cárdenas H, Caullet C, Chen SY, Chun YJ, Cossu C, Crane CF, Cros-Arteil S, Cudney-Bueno R, Danti R, Dávila JA, Della Rocca G, Dobata S, Dunkle LD, Dupas S, Faure N, Ferrero ME, Fumanal B, Gigot G, González I, Goodwin SB, Groth D, Hardesty BD, Hasegawa E, Hoffman EA, Hou ML, Jamsari AFJ, Ji HJ, Johnson DH, Joseph L, Justy F, Kang EJ, Kaufmann B, Kim KS, Kim WJ, Koehler AV, Laitung B, Latch P, Liu YD, Manjerovic MB, Martel E, Metcalfe SS, Miller JN, Midgley JJ, Migeon A, Moore AJ, Moore WL, Morris VRF Navajas M, Navia D, Neel MC, De Nova PJG, Olivieri I, Omura T, Othman AS, Oudot-Canaff J, Panthee DR, Parkinson CL, Patimah I, Pérez-Galdino CA, Pettengill JB, Pfautsch S, Piola F, Potti J, Poulin R, Raimondi PT, Rinehart TA, Ruzainah A, Sarver SK, Scheffler BE, Schneider ARR, Silvain JF, Siti Azizah MN, Springer YP, Stewart CN, Sun W, Tiedemann R, Tsuji K, Trigiano R, Vendramin GG, Wadl PA, Wang L, Wang X, Watanabe K, Waterman JM, Weisser WW, Westcott DA, Wiesner KR, Xu XF, Yaegashi

S, Yuan JS. 2009. Permanent Genetic Resources added to Molecular Ecology Resources database 1 January 2009-30 April 2009. *Molecular Ecology Resources* 9: 1375-1429.

Mun J, Song YH, Roderick GK. 2004. Isolation and characterization of microsatellites in the brown planthopper, *Nilaparvata lugens* Stal. *Korean Journal of Applied Entomology* 43: 311-315

Peakall R, Souse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288-295.

Raymond M, Rousset F. 1995. GENEPOP version 1.2. Population genetic software for exact tests and ecumenicism. *Journal of Heredity* 86: 248-249.

Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223-225.

Schlotterer C, Tautz D. 1992. Slippage synthesis of simple sequence DNA. *Nucleic Acids Research* 20: 211-215.

Schuler GD, Boguski MS, Stewart EA et al. 1996. A gene map of the human genome. *Science* 274: 540-546.

Squirrell J, Hollingsworth PM, Woodhead M, Russell J, Lowe AJ, Gibby M, Powell W. 2003. How much effort is required to isolate nuclear microsatellites from plants? *Molecular Ecology* 12: 1339-1348.

Thiel T, Michalek W, Varshney RK, Graner A. 2003. Exploiting EST database for the development and characterization of gen-derived SSR-markers in barley (*Hordeum*

vulgare L.). *Theoretical Applied Genetics* 106: 411-422.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins, DG. 1997. The CIUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tool. *Nucleic Acids Research* 25: 4876-4882.

Valdes AM, Slatkin M, Freimer NB. 1993. Allele frequencies at microsatellite loci-the stepwise mutation model revisited. *Genetics* 133: 737-749.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535-538.

Weng Y, Azhaguvel P, Michels Jr GJ, Rudd JC. 2007. Cross-species transferability of microsatellite markers from six aphid (Hemiptera: Aphididae) species and their use for evaluating biotypic diversity in two cereal aphids. *Insect Molecular Biology* 16: 613-622.

Wang D, Liao X, Cheng L, Yu X, Tong J. 2007. Development of novel EST-SSR markers in common carps by data mining from public EST sequences. *Aquaculture* 271: 558-574.

Zane L, Bargelloni L, Patarnello T. 2002. Strategies for microsatellite isolation: a review. *Molecular Ecology* 11: 1-16.

Table 1. The *Nilaparvata lugens* populations used in this study.

Population name	Site	location	Sampling dates	No. females	No. males
JS	Jiangsu province	119°26'E 32°08'N	Aug-12	0	45
ZJ	Zhejiang province	120°12'E 30°16'N	Aug-14	35	12
GX	Guangxi province	110°01'E 24°59'N	Aug-14	39	9

Table 2. Frequency and distribution of SSRs in the analyzed the *Nilaparvata lugens* ESTs.

Repeats	Number of repeat motif (n)											Total	
	5	6	7	8	9	10	11	12	13	14	15		>15
AC/GT	-	27	5	5	3	2	-	-	1	1	-	5	49
AG/CT	-	32	16	3	6	3	1	1	-	3	1	1	67
AT/AT	-	25	8	12	2	-	-	1	-	-	-	1	49
AAC/GTT	11	9	6	3	2	-	2	-	-	-	-	2	35
AAG/CTT	36	17	10	6	3	3	2	-	1	-	4	12	94
AAT/ATT	41	9	6	6	-	-	1	2	-	1	1	2	69
ACC/GGT	6	3	1	-	-	-	-	-	-	-	-	0	10
ACG/CTG	3	6	4	1	-	-	-	-	-	-	-	0	14
ACT/ATG	24	6	6	4	1	-	-	-	1	-	-	0	42
AGC/CGT	13	6	8	2	-	-	-	-	-	1	-	0	30
AGG/CTT	22	8	4	2	-	-	1	-	-	-	-	0	37
AGT/ATC	10	8	2	2	-	-	1	-	-	-	-	1	24
CCG/CGG	6	1	-	-	-	-	-	-	-	-	-	0	7
AAAG/CTTT	1	-	-	-	-	-	-	-	-	-	-	0	1
AAAT/ATTG	3	1	-	1	-	-	-	-	-	-	-	0	5
AACT/ATTG	1	1	-	-	-	-	-	-	-	-	-	0	2
AAGC/CGTT	-	-	-	-	-	1	-	-	-	-	-	0	1
AATC/AGTT	1	-	-	-	-	1	-	-	-	-	-	0	2
AATG/ACTT	1	-	1	2	1	-	-	-	-	-	-	0	6
AGAT/ATCT	2	-	1	1	-	-	-	-	-	-	-	0	5
AAAGT/ATTTC	1	-	-	-	-	-	-	-	-	-	-	0	1
AAATC/AGTTT	1	-	-	-	-	-	-	-	-	-	-	0	1
AATAG/ATCTT	1	-	-	-	-	-	-	-	-	-	-	0	1
AATGG/ACCTT	1	-	-	-	-	-	-	-	-	-	-	0	1
ACGTC/AGTGC	1	-	-	-	-	-	-	-	-	-	-	0	1
AAGGT/AGTTCC	1	-	-	-	-	-	-	-	-	-	-	0	1
NN(DNR)	-	84	29	20	11	5	1	1	2	4	1	7	165
NNN(TNR)	172	73	47	26	6	3	7	2	2	2	5	17	362
NNNN(TTNR)	9	4	2	4	1	2	0	0	0	0	0	0	22
NNNNN(PNR)	5	0	0	0	0	0	0	0	0	0	0	0	5
NNNNNN(HNR)	1	0	0	0	0	0	0	0	0	0	0	0	1

NN(DNR) are dinucleotidic repeats; NNN(TNR) are trinucleotidic repeats; NNNN(TTNR) are tetranucleotidic repeats; NNNNN(PNR) are pentanucleotidic repeats; NNNNNN(HNR) are hexanucleotidic repeats.

Table 3. Primer sequence and character of 12 microsatellite locus isolated in the *Nilaparvata lugens*.

Accession no.	Repeat motif	Primer sequence (5'-3')	No. of alleles and size range (bp)	Annealing temp. (°C)
DB828220	(TAT)8	F:CTTAAATTTGATGAAGTGAACCTG R:CTTGGTTGAATAATAAGGGATAT	6 (118-129)	55
DB840162	(TG)8	F:GGGAGAAGGAGGGAAGAGG R:CGAATGTGAAATGAGTGAGTGC	10 (204-224)	55
DB827752	(GA)7	F:GGAACTGCGCATGATGACA R:CTATGATTTGCTGTATTGTAAG	5 (231-262)	55
DB839890	(ATC)6	F:TTACCCTTCGGTTTCGC R:CCGATTATTTGTTGATGTTTGT	6 (165-180)	55
DB846024	(CCA)7caaccac caacatcg(ACC)6	F:GACCTGGTGTCCATTTTCGA R:GTGACTGAGCCTGGTCTGTG	4 (283-296)	55
DB839391	(CTC)6	F:GGGGCGAAAGAGAAGAGAG R:TCTACTGCTGATGGTGTGAAA	6 (158-173)	55
DB842630	(CTT)5	F:CAGATCATTGGCGGTTTCG R:GATAAGGATTGTGAGGGGAA	3 (124-130)	55
DB842620	(CGC)5	F:GCACACGCTTCTTTGCCG R:TGTGTTGAATGAATGAGTCTCATT	3 (203-209)	55
DB857195	(ATT)5	F:AAACAAAACCCAAAACACA R:GAGGATAAAAAGAAAATCAACAA	3 (215-223)	55
DB845048	(GAA)5	F:CACGCTGGAGCCTGTGA R:CTGCTTCTGTGCAATTTGAAAT	3 (103-109)	55
DB844949	(GAA)5	F:GACATCGTCGCTCTGGCT R:CCGTTGTGGTAATACCTTTTGGC	2 (311-314)	55
DB830423	(AG)6	F:AGTTCAAGGCTAACGTGAACCT R:GACAGAACAGAAAAAGAAATGGA	5 (171-185)	55

Table 4. Population genetic parameters for each locus and population of the *Nilaparvata lugens*.

Population		Locus												Mean
		NL22	NL41	NL74	NL121	NL122	NL140	NL157	NL158	NL161	NL162	NL167	NL177	
ZJ	N	47	47	47	47	46	47	46	46	47	47	46	46.667	
	A	4	11	5	5	5	7	1	4	3	3	6	4.75	
	Ho	0.702	0.83	0.255	0.383	0.63	0.723	0	0.304	0.277	0.511	0.383	0.5	0.458
	He	0.513	0.819	0.301	0.574	0.523	0.692	0	0.356	0.333	0.632	0.517	0.689	0.496
	Null	-0.279	-0.006	0.053	0.166	-0.124	-0.017	0	0.058	0.097	0.083	0.117	0.13	
GX	HWE	-	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	
	N	48	48	48	48	48	48	48	48	48	48	47	47.917	
	A	4	10	5	8	5	7	2	4	3	4	3	5.167	
	Ho	0.458	0.667	0.271	0.521	0.5	0.75	0.042	0.188	0.458	0.313	0.511	0.563	0.437
	He	0.403	0.659	0.241	0.557	0.501	0.792	0.041	0.243	0.436	0.478	0.49	0.696	0.461
JS	Null	-0.118	-0.007	-0.145	0.022	-0.038	0.027	-0.022	0.086	-0.035	0.149	-0.013	0.081	
	HWE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
	N	45	44	45	45	45	45	45	45	44	45	45	44.833	
	A	6	10	5	6	4	6	3	3	3	2	5	4.667	
	Ho	0.267	0.818	0.244	0.489	0.267	0.644	0.111	0.289	0.432	0.333	0.511	0.244	0.388
Total all populations	He	0.411	0.815	0.224	0.663	0.287	0.706	0.182	0.255	0.503	0.367	0.496	0.533	0.454
	Null	0.178	0.026	-0.12	0.126	0.039	0.029	0.118	-0.145	0.068	0.068	-0.037	0.242	
	HWE	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	
	Mean all populations	Ho	0.476	0.772	0.257	0.464	0.466	0.706	0.051	0.26	0.389	0.386	0.468	0.436
	He	0.442	0.764	0.255	0.766	0.437	0.73	0.074	0.285	0.424	0.492	0.501	0.639	
PIC	N	140	139	140	140	139	140	139	139	140	139	139		
	A	6	13	9	8	6	8	3	4	4	5	3	9	
	PIC	0.41	0.807	0.492	0.57	0.421	0.722	0.074	0.45	0.532	0.453	0.39	0.714	0.503

N is number of analyzed individuals; A is number of alleles detected; Ho is observed heterozygosity; HE is expected heterozygosity; PIC is polymorphism information content; Null is frequency of null allele; HEW is Hardy-Weinberg equilibrium (HEW) test. Values that are significant after Sequential Bonferroni correction for multiple tests are indicated by * ($p < 0.05$).