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Isolation and characterization of polymorphic microsatellite loci in the bamboo locust *Rammeacris kiangsu* (Orthoptera: Acrididae)

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Abstract. Twelve polymorphic microsatellite loci were developed and characterized from the bamboo locust, *Rammeacris kiangsu,* based on enriched genomic libraries. Analysis of 30 individuals showed that the number of alleles ranged from seven to 25 with the observed heterozygosity ranging from 0.333 to 0.767 and expected heterozygosity from 0.784 to 0.963. Test of cross-species amplification showed that some of these microsatellite markers could be used for studying other species such as *Ceracris nigricornis*, *C. fasciata*, and *Chorthippus brunneus.*

INTRODUCTION

The bamboo locust, *Rammeacris kiangsu*, is one of the most important pests of bamboos in China (Li et al., 1998; Zhang et al., 2007). The nymphs and adults of this locust mainly feed in large groups on the leaves of bamboo plants, often causing new culms to die and a decrease in the production of new shoots (Xu & Wang, 2004). Great efforts have been made to find effective strategies and methods for controlling this species (Shen et al., 2009). Here we describe the development of twelve microsatellite loci from *R. kiangsu*. Because of their high polymorphism and co-dominance these loci will be useful as molecular markers for determining the levels of genetic diversity in bamboo locust populations.

MATERIAL AND METHODS

Genomic DNA was extracted from ethanol-preserved samples of four unrelated individuals using a standard proteinase K/phenol extraction protocol (Sambrook & Russell, 2001). Microsatellite loci were obtained using the FIASCO (fast isolation by AFLP of sequences containing repeats) protocol described by Zane et al. (2002) with slight modifications. One microgram of *Mse*I adaptor (5'-TACTCAGGACTCAT-3'/5'-GACGATGAGTCCTGAG-3') was ligated to approximately 250 ng of genomic DNA after digestion with the restriction enzyme, *Mse*I (BioLabs, Beijing, China). The digestion-ligation mixture was diluted (1 : 10) and amplified with adaptor-specific primers (5'-GATGAGTCCTGAGTAAN-3', *Mse*I-N) in a total volume of 20 µL reaction containing: $MgCl_2$ 1.5 mM, *Mse*I-N 0.5 µM, dNTPs 250 µM, 1 U of *Taq* DNA polymerase (TaKaRa, Dalian, China) and 5 µL diluted digestion-ligation DNA. The PCR conditions were 5 min at 94°C followed by 20 cycles of 30 s at 94°C, 1 min at 53°C, 1 min at 72°C with a final extension time of 10 min at 72°C. After denaturation of 5 min at 95°C, amplified product was hybridized with biotinylated $(GA)_{12}$ and $(TGTA)_6$ for 1 h at 68°C, respectively. DNA fragments hybridized to biotinylated probes were selectively captured by streptavidin-coated magnetic beads (Streptavidin Magnesphere Paramagnetic Particles, Promega, Shanghai, China), following the procedure presented by Zane et al. (2002). Then, nonspecific binding and unbound DNA was removed by several non-stringent and stringent washes.

These microsatellite-enriched DNA fragments were purified using EZ-10 Spin Column PCR Products Purification Kit (BIO BASIC INC., Shanghai, China) and then amplified for 35 cycles using *Mse*I-N primers. The PCR products were ligated into pGEM-T Easy vectors (Promega) and transformed into *Escherichia coli* strain (DH5α). Transformed cells were cultivated at 37°C for about 16 h on LB agar plate containing ampicillin, X-gal, and IPTG for blue/white selection. Insert-positive bacterial clones were amplified using M13 primers and visualized by agarose gel electrophoresis. Ninety-five positive clones were screened and sequenced.

Fifty-six primer sets were designed through Primer 3 software (Rozen & Skaletsky, 2000) and synthesized. Fluorescent dye labelling of PCR fragments was performed with three primers: a sequence-specific forward primer or reverse primer with M13 tail at its 5' end (5'-CACGACGTTGTAAAACGAC-3'), a sequence-specific reverse primer or forward primer and the fluorescent-labelled M13 primer (either IRD700 or IRD800, LI-COR). PCR amplifications were carried out in 15 µL volumes containing 50 ng template DNA, $MgCl_2$ 1.5 mM, dNTPs 250 µM, 0.75 U of *Taq* DNA polymerase (TaKaRa), 1×PCR buffer, 2.5 pmol of each primer and 0.5 pmol of fluorescently labelled M13 primer [either IRD700 or IRD800 (LI-COR, Nebraska, USA)]. The amplifications included an initial denaturing at 95°C for 5 min, followed by 35 cycles of 30 s at 95°C, 30 s at 52–65°C depending on the primer pair (Table 1), and 30 s at 72°C, followed by a final extension step for 10 min at 72°C. PCR products were separated on 6.5% polyacrylamide gels using a LI-COR 4300 automated DNA sequencer and analysed using LI-COR saga ^{GT} software.

RESULTS AND DISCUSSION

The primers synthesized as described above were used for screening microsatellite polymorphism in 30 individuals collected from four different local populations (Jiangsu province, Chongqing municipality, Guangxi autonomous region, and Guizhou province). Genomic DNA from these 30 individuals was extracted using standard overnight proteinase K digestion,

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TABLE 1. Characteristics of the twelve polymorphic microsatellite loci isolated from bamboo locust.

Locus accession no.	Repeat motif	Primer sequence (5'–3')	Size range (bp)	Ta (°C)	Total			Pop1		Pop2		Pop3	
					N_A	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E
WJ621 GQ267818	$(AG)_{28}$	F: **M13-**CGGAGTGTTTCGCCTTCT R: ACGGGACTCGTTTACTACCA	243–283	57	16	0.767	0.899	0.800	0.853	0.800	0.916	0.700	0.932
WJ622 GQ267819	$(GA)_{27}$	F: **M13-**GCAGGATGACCTTTTGAG R: TGCTTCGAGTCACTGTTC	173–205	55	14	0.600*	0.860	0.800	0.889	0.500	0.753	0.500	0.847
WJ623 GQ267820	$(GA)_{21}$	F: **M13-**CAGTCCTTGCTCAACCGT R: CAAAGGCGGTGGCATAAT	257–281	60	12	0.533*	0.884	0.400	0.805	0.800	0.905	0.400	0.826
WJ624 GQ267821	$(TG)_{19}(AG)_{23}$	F: **M13-**CGTTTCGTCGTCGAGGTAAAAT R: CCGCTGCTATAATGATCAAGGGA	166–212	52	19	0.600	0.946	0.500	0.921	0.800	0.963	0.500	0.942
WJ625 GQ267822	$(GA)_7T(AG)_{20}$	F:**M13-**TCAATACATCTTGCTGCTACGCG R: GCCTATCAATCACTGCCCCATC	201–253	55	20	0.467*	0.945	0.500	0.942	0.700	0.953	0.200	0.848
WJ626 GQ267823	$(GA)_{20}T(AG)_5$ $(CG)_2A(AG)_{19}$	F:**M13-**CGGGTTTGTAATAGATGGTTGTCC R: GGCGTGGTCTGTAATTTCAGAAG	200–256	59	25	0.700	0.963	0.700	0.816	0.800	0.963	0.600	0.947
WJ627 GQ267824	$(AG)_{27}$	F: **M13-**CTTGAGGACGACACCGCATTG R: CTCCCAATAACCCGCATCAGA	213–241	65	11	0.567*	0.898	0.600	0.816	0.600	0.926	0.500	0.884
WJ628 GQ267825	$(CT)_{16}CA(CT)_2$ $ACGG(AC)_{10}$	F: **M13-**GAGGGACTGATGACCTTAGCA R: CGGACCCACGTAACTACAGACT	211–245	62	11	0.333*	0.829	0.800	0.847	0.100	0.868	0.100*	0.732
WJ629 GQ267826	$(GA)_{23}$	F: CGTGCTTTGGTTCATGGGGTTA R: **M13-**TGCTGTCACATCGGATCTTCG	198–232	64	14	0.600*	0.905	0.700	0.837	0.600	0.821	0.500	0.895
WJ630 GQ267827	$(TG)_{24}A(GT)_2$ $(GA)_8A(AG)_{15}$	F: CGCTTCTGTAGGAGCTTTCTAAC R: **M13-**GTCAGTCTAGGGATCGATGACC	185–207	60	10	0.600*	0.860	0.400	0.858	0.700	0.768	0.700	0.905
WJ631 GQ267828	$(TGTA)_2TA$ $TG(TGTA)_6$	F: **M13-**GCATAGGAACGCACAGTAG R: GTAACCCCACAGCGATTG	241–265	60	11	0.667*	0.786	0.600	0.821	0.700	0.537	0.700	0.900
WJ632 GQ267829	$(TG)_5(TATG)_6$ $(CATG)_3$	F: **M13-**GCGTGTACCCTAGTGATGC R: TACCTGCTGGGCTAATGTG	214–242	55	7	0.600*	0.788	0.800	0.784	0.500	0.732	0.400	0.821

Pop1, Chongqing population (n = 10); Pop2, Jiangsu population (n = 10); Pop3, Guangxi population (n = 8), and Guizhou population (n = 2). Ta, optimal annealing temperature; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity. Significant deviations from Hardy-Weinberg equilibrium after sequential Bonferroni correction at $P < 0.05$ are marked with an asterisk. Primer sequences with '**M13-**' indicate M13F (-29) (5'-CACGACGTTGTAAAACGAC-3') that was added to the 5'-end of the primer.

TABLE 2. Results obtained from cross-species amplification tests on two *Ceracris* species and two species of the genus *Chorthippus*.

Locus	WJ621	WJ622	WJ623	WJ624	WJ625	WJ626	WJ627	WJ628	WJ629	WJ630	WJ631	WJ632
Ceracris nigricornis	–	+	+	+	+	+	–	–	–	+	–	–
Ceracris fasciata	–	–	–	–	+	–	–	–	–	+	–	–
Chorthippus brunneus	–	–	–	–	+	–	–	+	–	–	–	+
Chorthippus changbaishanensis	–	–	–	–	+	–	–	+	–	–	–	+

+, successful amplification; –, unsuccessful amplification.

followed by phenol-chloroform extraction and ethanol precipitation (Sambrook & Russell, 2001). Of the 56 primer pairs tested, 24 successfully amplified the target regions, but only 12 of them revealed microsatellite polymorphisms. The number of alleles at each polymorphic locus, their size range, and observed and expected heterozygosities were calculated using Cervus 2.0 software (Marshall et al., 1998). The results are shown in Table 1. The number of alleles per locus ranged from 7 to 25. These microsatellite loci showed high levels of polymorphism. Total observed and expected heterozygosities ranged from 0.333 to 0.767 and 0.722 to 0.963, respectively. Deviation from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium at each locus was calculated using GenePop 3.4 (Raymond & Rousset, 2004). No significant linkage disequilibrium was detected among the twelve loci. After sequential Bonferroni correction (Rice, 1989), most of the loci (WJ622, WJ623, WJ625, WJ627, WJ628, WJ629, WJ630, WJ631, and WJ632) showed significant deviations from Hardy-Weinberg equilibrium. The use of Microchecker (van Oosterhout et al., 2004) indicated that this phenomenon may be the result of null alleles. However, this may also be attributable to a Wahlund effect (Wahlund, 1928). The Wahlund effect may result in heterozygote deficiency that is actually caused by subpopulation structure, and is the most likely explanation of the results presented here. In addition, the small sample size was an important reason for the low power of the tests for linkage and HW equilibriums.

In order to assess interspecific amplification, cross-species amplification was tested in four other locusts, two *Ceracris* species and two species from the genus *Chorthippus* (Table 2). DNA samples from four individuals of each species were tested, using the same PCR conditions as for *R. kiangsu.* Nine of the twelve loci (except WJ621, WJ627, and WJ631) can be amplified successfully in at least one other species, with one loci (WJ625) amplified in all species tested. The results suggest that the microsatellites isolated from *R. kiangsu* can be useful, specially half of them, to conduct population genetic studies on *Ceracris nigricornis*, but their potential for cross-species amplification within Acrididae is limited.

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