Development and Isolation of 17 polymorphic Microsatellite Loci in *Coptoterms formosanus* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Seventeen polymorphic microsatellite DNA loci for *Copototermes formosanus* were isolated and characterized. Polymorphism of these loci was assessed in a sample of 32 unrelated *C. formosanus* individuals. An average of 4.6 alleles per locus (3-8 alleles) was detected. Observed and expected heterozygosities ranged from 0.2500 to 1.0000 and from 0.5591 to 0.8562, respectively. Six loci were found to have deviated from Hardy-Weinberg equilibrium in the sampled population after Bonferroni correction. No significant linkage disequilibrium was detected. These markers will be useful in population genetics, phylogenies and other relevant studies of *C. formosanus.*

Key words**:** *Coptotermes Formosanus*, Microsatellite loci, Polymorphism

INTRODUCTION

The Formosan subterranean termite (FST) *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) is a major economic pest worldwide. It is thought to have originated in China, but has spread to many areas around the world where it is a highly destructive pest of wood structures (Su & Tamashiro 1987). Recently, mitochondrial DNA sequencing (Jenkins *et al*. 2002; Austin *et al*. 2006; Fang *et al*. 2008) has been used to study the genetic diversity of *C. formosanus,* mainly in China, America and Japan, but this information is insufficient for investigating the genetic diversity and detailed spatial structure of these populations. Microsatellite DNA loci as polymorphic genetic markers are necessary for elucidating the details of colony organization, population structure, and relationships among introduced and native populations (Ross

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2001, Vargo *et al.* 2003). Although Vargo & Henderson (2000) identified 12 microsatellite loci in *C. formosanus*, few subsequent studies have been performed. Therefore we isolated and characterized 17 polymorphic microsatellite loci for *C. formosanus* following the protocol modified from Yu *et al.* (2010), so as for further study of population genetics, phylogenies and other relevant study of *C. formosanus*.

MATERIALS AND METHODS

Total genomic DNA was extracted from the heads of twenty *C. formosanus* individuals, using the regular phenol-chloroform method as described by Zhang and Hewitt (1998). Approximately 4 ug high quality genomic DNA was digested with restriction enzyme *Sau* 3AI (Promega), and the size-selected DNA (400 - 900 bp) was isolated from an agarose gel using DNA purification kit (TaKaRa). Fragments were then ligated to a blunt-end adapter (SAULA: GCGGTACCCGGGAAGCTTGG, SAULB: GATCCCAAGCTTC-CCGGGTACCGC) with T4 DNA ligase (Takara) at 16°C for 14h. The ligation products were amplified by polymerase chain reaction using the adapter SAULA as primers. After denaturation at 95°C for 10 min, microsatellite-bearing amplified fragments were selected with the biotin-labeled $(AC)_{12}$ and $(AGG)_8$ (Sangon) oligonucleotide probes in sodium phosphate buffer (0.5 M sodium phosphate, 0.5% SDS, pH 7.4) at 50°C for 16h. The target fragments were amplified by PCR and the size-selected DNA (400 - 900 bp) were excised from agarose gel and recovered. These recovered DNA product were ligated with pMD19-T vector (TaKaRa) and transformed to *E. coli* DH5a competent cells (TaKaRa). Eighty-two positive clones were identified from 196 recombinant colonies via PCR with the adapter SAULA as primers, and were sequenced with M13 primers in one direction.

Forty-eight sequences contained microsatellites, of which thirty-five possessed sufficient flanking sequences appropriate for primer design. The others had either insufficient or inappropriate flanking regions on one or both sides of their microsatellites. These thirty-five pairs of primers were designed by the Premier 5.0 program (PREMIER Biosoft International, Silicon Valley, USA), and synthesized by Sangon Biotech (Shanghai) Co., Ltd.

A range of annealing temperatures (50 °C - 68 °C) were tested and the

temperature producing the cleanest and strongest PCR product when observed on an 1.5% agarose gel stained with Goldview was selected for PCR. After these optimization procedures, seventeen of the thirty-five primer sets designed amplified successfully and were used to assess polymorphism based on 32 unrelated *C. formosanus* individuals.

Following Genomic DNA extraction, PCR was performed in a volume of 20 ul containing 50 - 100 ng of total DNA, 0.25 - 0.5 units of Taq polymerase (Tiangen, China), 19 PCR buffer, 1.0 - 2.0 mM $MgCl_2$, 0.2 mM dNTPs, and 0.2 - 1 uM of each primer. The PCR profile was as follows: initial denaturation at 95 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, a primer-specific annealing temperature for 30 s and 72 °C for 40 s, with a final extension at 72 °C for 10 min. The PCR products were checked by electrophoresis on 8% non-denaturing polyacrylamide gel, and visualized with silver staining. Allele size was determined against a 50-bp DNA ladder (Tiangen, China) with software Gel-Pro Analyzer 4.5. Number of alleles, heterozygosity, test of Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were analyzed using GENEPOP4.0.6 (Raymond and Rousset 1995).

RESULTS

Conditions and characteristics of the 17 loci are shown in Table 1. The number of observed alleles per locus ranged from 3 to 8. The expected and observed heterozygosity values ranged from 0.2500 to 1.0000 and 0.5591 to 0.8562, respectively. Six loci (Copf02, Copf04, Copf09, Copf10, Copf13 and Copf17) significantly deviated from Hardy-Weinberg equilibrium after Bonferroni correction ($P<0.0029$). No significant linkage disequilibrium was detected. We conclude that the markers obtained will be useful in population genetics, phylogenies and other relevant study of *C. formosanus*.

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Table 1 Characterization of 17 polymorphic *Coptotermes formosanus* microsatellite loci. Motif, repeat sequence of the isolated clone; *Ta*, annealing temperature; *A*, the number of alleles; *Ho*, the observed heterozygosity; *He*, expected heterozygosity and *P*, associated probability value of conformation with Hardy–Weinberg equilibrium (HWE).

Locus	Accession no.	Primer sequence (5'–3')	Motif	*Ta* (°C)	Allete size (bp)	*A*	*Ho*	*He*	*P*
Copf01	JQ313794	ATTCCTTCACTTACGCACTT GTACCCGACATCATACGC	(CT)10TT(CT)8	65	297	7	0.4074	0.7932	0.0113
Copf02	JQ313795	GGAAGAAGGACCAATCTG TAACCAAGGAGCGTAATG	(GT)8	62	190	4	0.9688	0.6652	0.0000
Copf03	JQ313796	ACCGACTCCTCTGATTGA CACATTATGTTTCCACGAC	(GT)22（GA）9	64	184	5	0.5806	0.5711	0.0069
Copf04	JQ313797	TACCGGACTCTAACAGACA TCAGAGGATTCTTACCGA	(AC)7TC(AC)5	62	360	3	0.3125	0.7287	0.0000
Copf05	JQ313798	GCAATGAAGTGCCTCTGA AACCTGGACTCGACCTTT	(AC)19	65	261	8	0.8438	0.6935	0.0827
Copf06	JQ313799	CAGTGGCAGCGACGTATA ATCCTGGAGTCCTAAGAAGC	(AC)8GC(AC)14	65	186	6	0.2500	0.6696	0.0196
Copf07	JQ313800	CTCTTTGCTGCCATACGT CTCAGTTCCATGCGGACA	(GT)18	65	242	5	1.0000	0.5591	0.0512
Copf08	JQ313801	TCAATGGCGTGCCTTCAC AGCTCAACCACTGCGTTT	(CACT)13(CATT)16	62	263	6	0.9688	0.7277	0.1835
Copf09	JQ313802	GTGCTGGCGTTCGGTATT TTTGCTTGCCTAAAGTCG	(AC)8N(AC)6N(AC)6	62	257	4	0.8750	0.8562	0.0001
Copf10	JQ313803	AGGTGTTGAATGGGCTGTT CCAAGCCTGCCAGAAAGT	(AC)17	65	333	3	1.0000	0.7142	0.0000
Copf11	JQ313804	CGAAGTTATGCCTCTGTT TTTGGATGCCTGGATTAG	(AC)8	62	286	4	0.8710	0.6785	0.0345
Copf12	JQ313805	GTGCTGGAGTTTGGATTT GAGGCGGTAGTAACAATAAG	(GT)6N(GT)6	62	334	3	0.8333	0.6870	1.0000
Copf13	JQ313806	TATTGTTGTTGCGGAAGC GTCGGCAGCACTGAAGTA	(GT)13	62	196	5	0.3929	0.6714	0.0000
Copf14	JQ313807	CTACAAGGCTACCATCAGG GGAACAGCGAGACGAGAT	(CT)13	64	222	4	1.0000	0.7821	0.5283
Copf15	JQ313808	TCTCCGTTCATCACAGCC CAGGGAAAGCAACCACATC	(GT)16	65	323	4	0.7037	0.7659	0.1070
Copf16	JQ313809	CGTCACGTTATGGAGCAT AGCGGACTTGAGGTTAGA	(GT)16	64	267	4	0.9259	0.6981	0.0945
Copf17	JQ313810	TGTTTCACAGCCATCAGA TGCTTGGTAAATGGGTAG	(AC)12	62	184	4	1.0000	0.5714	0.0000

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