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SHORT NOTE

Isolation and Characterization of Polymorphic Microsatellite Markers for Two Subterranean Termites

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Abstract
We isolated 15 and 18 highly polymorphic genomic microsatellite markers from two subterranean termites, *Reticulitermes aculabialis* and *R. labralis*, respectively. A total of 53 alleles were detected in 15 microsatellite loci of *R. aculabialis*, and the alleles were 3.533±1.302 (mean±SD), while the corresponding data of *R. labralis* were 115 detected alleles in 18 microsatellite loci with 6.389±1.754 alleles. The observed and expected heterozygosity was 0.496±0.236 and 0.564±0.125 in *R. aculabialis*, and 0.368±0.263 and 0.702±0.115 in *R. labralis*, respectively. Seven loci were highly polymorphic (PIC>0.5) in *R. aculabialis*, and 15 loci were highly polymorphic (PIC>0.5) in *R. labralis*. All loci showed Hardy–Weinberg equilibrium. These polymorphic markers provide useful tools for population genetic and breeding system studies of subterranean termites.

Subterranean termites are the most common and economically important species (Nobre et al., 2006; Pinzon et al., 2009). Many termite species have become successfully established outside their native ranges, and cause environmental and economic damages to the invaded areas (Perdereau et al., 2011). *Reticulitermes aculabialis* and *R. labralis* are two important subterranean termites, particularly in the middle and eastern China, where they are considered invasive (Su et al., 2016; Wang et al., 2016). Although the population status and the breeding structure of several termite species have been analyzed by microsatellite loci (Vargo & Carlson, 2006; Vargo et al., 2013; Huang et al., 2013; Perdereau et al., 2015), highly polymorphic microsatellite markers used for analysis of *Reticulitermes* termites are still insufficient. To facilitate the research of termite genetic diversity and population structure, we have screened 147 microsatellites and identified 15 and 18 highly polymorphic loci for *R. aculabialis* and *R. labralis*, respectively.

The samples of *R. aculabialis* and *R. labralis* were both collected from eight locations of Xi'an, China. For each location, two worker samples were randomly selected to detect the polymorphism. In order to exclude the intestinal microbes in the termite abdomen, we extracted DNA from the head of the termite. The genomic sequence library and microsatellite library were designed and set up according to the DNA of *R. aculabialis*. The constructed library obtained a number of 100 bp short sequence fragments by Illumina Hiseq 2000 sequencing. We used default parameter settings of CLC Genomics Workbench V7.5 software to trim the fragments with excellent quality and *de novo* assemble them. Only the consensus sequences were used for microsatellite scanning. By scanning the microsatellite loci of above consensus sequences

with SciRoKo V3.4 software (Kofler et al., 2007), a total of 594 microsatellite loci were found, distributed on 571 consensus sequences. 147 consensus sequences were randomly selected among the above 571 sequences and designed by P_{RIMER} V3.0 (Rozen & Skaletsky, 2000). The primers were synthesized by Genedigger (Xi'an) Technology Co., Ltd.

DNA was extracted using TIANamp Genomic DNA Kit, and the extracted DNA were amplified with a thermocycler (Mastercycler nexus gradient) in 5 μL reactions containing 2×Taq PCR Mix 2.5 μL, ddH_2O 1.1 μL,0.2 μL of each primer and 1 μL DNA. The amplification program consisted of an initial denaturation at 94°C for 5 min followed by 30 cycles at 94°C for 30 sec, annealing temperature (*Ta* in Table1and Table 2) for 30 sec and 72°C for 30 sec, final extension at 72°C for 10 min. Amplification products were analyzed with polyacrylamide gel electrophoresis and genotyped using Quantity Quantity One software. The number of alleles (*k*), observed heterozygosity (H_O), expected heterozygosity (H_E) and Hardy-Weinberg equilibrium (HWE) test were performed by $G_{EN}A_{LEX}$ V6.501, and polymorphism information content (PIC) were estimated by C_{ERVUS} V3.0 (Kalinowsk etal., 2007).

All loci met with Hardy–Weinberg equilibrium. The 147 loci were screened both in *R. aculabialis* and *R. labralis*, with an isolation of 22 pairs and 21 pairs microsatellite primers in these two species, respectively. All these primers yielded stable and effective amplification bands. Then, 15 highly polymorphic microsatellite loci in *R. aculabialis* and 18 highly polymorphic microsatellite loci in *R. labralis* were identified. For the 15 polymorphic loci of *R. aculabialis*, 8 are moderately polymorphic (0.25<PIC<0.5), 7 are highly polymorphic (PIC>0.5) (Table1). For the 18 polymorphic loci of *R. labralis*, 3 are moderately polymorphic (0.25<PIC<0.5), 15 are highly polymorphic (PIC>0.5) (Table2). We calculated the genetic diversity per locus, i.e number of alleles (*k*), observed heterozygosity (H_O), expected heterozygosity (H_E) and PIC. Number of alleles (*k*) for each locus ranged between 2 and 6 in *R. aculabialis*. The mean alleles were 3.533±1.302 (mean±SD). Whereas the observed and expected heterozygosity

Table 1. Highly polymorphic microsatellite primers in *Reticulitermes aculabialis.*

Locus	Primer sequence (5'–3')	*k*	Size	Motif	*Ta*	H_O	H_E	PIC
Ra014	F:CGTACTGCGGGAAGTACTGA R:TGTTGTGCTTTAGTGCTGGC	4	259-284	$(AATTC)_5$	51.8	1.000	0.711	0.658
Ra022	F:ACAGATCAGACGCAAGGCTC R:AGATGATGATGCTGGGCTCT	3	213-235	$(AGGC)_6$	55.6	0.938	0.607	0.539
Ra024	F:AGAAGGACTCTGCTGCATGG R:CGTTGTAACCACATGCCAAG	2	180-184	$(AGTT)_{10}$	55.6	0.500	0.492	0.371
Ra050	F:TCCAGTTGTCACTTCGACAGA R:GTCAAGGTCCCGTCCTGTTA	4	107-119	$(ATGT)_{15}$	50.3	0.438	0.525	0.486
Ra070	F:TACAGAGCTTTCATGGCACG R:AAACCTCGAAATGAGGAGGC	3	150-156	$(CTA)_{12}$	58.2	0.500	0.607	0.530
Ra079	F:TACCCTGTGGAGAACTCGCT R:AATGACCTTCTTGGGCGTTT	3	200-208	$(GAAT)_9$	55.6	0.250	0.508	0.428
Ra095	F:CTGCTAGGAAGCAACGAACC R:AAGACCTCGGAAAGAGGAGG	3	160-163	$(TAA)_9$	59.1	0.375	0.389	0.334
Ra096	F:TCGTACATACAGACGGACGTG R:GCTTCTCAAGAAGGACTGTGC	3	212-232	$(TAAC)_{10}$	59.1	0.250	0.406	0.371
Ra098	F:ACAGCTTACGCCGCTGTATC R:CTCAAGAAGGACTCTGCTCCA	2	233-237	$(TAAC)_6$	59.1	0.313	0.451	0.349
Ra103	F:TGCCTGTTTCGTTGATGAAG R:ATCCAATCCTACTTGCGTGG	2	241-245	$(TACA)_{11}$	50.3	0.438	0.498	0.374
Ra116	F:TCGACCGACTCAGTAGCCTT R:AAAGATGGAGGGACGAGGTT	6	201-219	$(TCT)_{11}+(CCT)_6$	59.1	0.500	0.766	0.727
Ra128	F:GTCTCGTCAAATTGTTGGCA R:ATCACCGTTGGTTCAAGAGG	4	105-114	$(TTA)_{10}$	51.8	0.438	0.443	0.402
Ra130	F:AAAGAGGAGGCAAGAGGAGG R:CATCTCTGCGGTGATGAGAG	5	225-246	$(TTA)_{11}$	56.9	0.313	0.717	0.676
Ra132	F:GATTGGTTTCCTCCGAATCA R:AAAGACTACTGCCACCGGG	3	201-213	$(TTA)_{14}$	58.2	0.375	0.602	0.516
Ra144	F:CAAATAGAGCTCCGTGTTTCG R:CCATAGAAACCTCCGAAAGG	6	146-186	$(TTAG)_7$	56.9	0.813	0.736	0.698

Ta the annealing temperature, *Size* approximately product size, *k* number of alleles, *HO* observed heterozygosity, *HE* expected heterozygosity, *PIC* polymorphism information content.

ranged between 0.250 to 1.000 and 0.389 to 0.766, respectively. The mean observed and expected heterozygosity were 0.496±0.236 and 0.564±0.125, respectively. The PIC varied from 0.334 to 0.727 with a mean of 0.497±0.137. In addition, number of alleles (*k*) ranged from 3 to 9 in *R. labralis*. The mean alleles were 6.389±1.754. Whereas the observed and expected heterozygosity ranged between 0.063 to 1.000 and 0.447 to 0.859, respectively. The mean observed and expected heterozygosity were 0.368±0.263 and 0.702±0.115, respectively. The PIC ranged from 0.371 to 0.843 with a mean of 0.663±0.127.

These results showed that the genetic variation of *R. aculabialis* and *R. labralis* were very high, suggesting that these microsatellite markers are essential for estimating the genetic diversity and population genetic of *Reticulitermes* termites.

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Disclosure

The authors declare no conflict of interest.

Table 2. Highly polymorphic microsatellite primers in *Reticulitermes labralis*.

Locus	Primer sequence (5'–3')	*k*	Size	Motif	*Ta*	H_O	H_E	PIC
Ra014	F:CGTACTGCGGGAAGTACTGA R:TGTTGTGCTTTAGTGCTGGC	7	260-290	$(AATTC)_5$	51.8	0.438	0.635	0.603
Ra022	F:ACAGATCAGACGCAAGGCTC R:AGATGATGATGCTGGGCTCT	6	212-244	$(AGGC)_6$	55.6	0.500	0.590	0.548
Ra024	F:AGAAGGACTCTGCTGCATGG R:CGTTGTAACCACATGCCAAG	6	156-184	$(AGTT)_{10}$	55.6	0.313	0.721	0.677
Ra050	F:TCCAGTTGTCACTTCGACAGA R:GTCAAGGTCCCGTCCTGTTA	9	160-232	$(ATGT)_{15}$	50.3	0.500	0.859	0.843
Ra070	F:TACAGAGCTTTCATGGCACG R:AAACCTCGAAATGAGGAGGC	7	144-174	$(CTA)_{12}$	58.2	0.313	0.723	0.695
Ra071	F:GAACAATGGTCATCCAGCCT R:TTGGCTATTCAGTCAGCACA	8	252-285	$(CTA)_8$	59.1	0.313	0.760	0.728
Ra079	F:TACCCTGTGGAGAACTCGCT R:AATGACCTTCTTGGGCGTTT	6	184-212	$(GAAT)_9$	55.6	0.063	0.666	0.635
Ra091	F:AACTTCCTTTGAATGCGCTC R:GCATACCAGAGGTCCTGCAT	9	233-261	$(TAA)_5$	56.9	0.188	0.846	0.828
Ra095	F:CTGCTAGGAAGCAACGAACC R:AAGACCTCGGAAAGAGGAGG	4	153-162	$(TAA)_9$	59.1	0.313	0.580	0.493
Ra096	F:TCGTACATACAGACGGACGTG R:GCTTCTCAAGAAGGACTGTGC	5	196-232	$(TAAC)_{10}$	59.1	0.250	0.500	0.474
Ra097	F:CACTGCAAGACGCAAAGTGT R:GCTTCTCAAGAAGGACTGTGC	4	252-264	$(TAAC)_5$	59.7	0.375	0.729	0.677
Ra103	F:TGCCTGTTTCGTTGATGAAG R:ATCCAATCCTACTTGCGTGG	5	232-252	$(TACA)_{11}$	50.3	0.125	0.729	0.682
Ra116	F:TCGACCGACTCAGTAGCCTT R:AAAGATGGAGGGACGAGGTT	8	177-219	$(TCT)_{11}+(CCT)_6$	59.1	1.000	0.752	0.721
Ra126	F:GTGCCGTTAGTTTGCCATTT R:AGTGGGAGCCGAGTTGTTC	3	216-224	$(TGTC)_7$	59.1	0.063	0.447	0.371
Ra128	F:GTCTCGTCAAATTGTTGGCA R:ATCACCGTTGGTTCAAGAGG	6	115-139	$(TTA)_{10}$	51.8	0.125	0.695	0.644
Ra130	F:AAAGAGGAGGCAAGAGGAGG R:CATCTCTGCGGTGATGAGAG	8	216-249	$(TTA)_{11}$	56.9	0.563	0.789	0.759
Ra141	F:CACATTTGAGGTTCGCAAGA R:GCCAGAAGGCCAATTACAGA	6	165-210	$(TTA)_8$	56.9	0.250	0.813	0.785
Ra144	F:CAAATAGAGCTCCGTGTTTCG R:CCATAGAAACCTCCGAAAGG	8	148-184	$(TTAG)_7$	56.9	0.938	0.805	0.779

Abbreviations as in table 1.

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