Study on the Genetic Diversity of Reticulitermes Aculabialis

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Abstract: Termites are a kind of insect pest that is harmful to the environment. The genetic diversity of two natural populations in Xi'an and Nanjing was investigated using 6 pairs of highly polymorphic microsatellite markers. The results showed that the average number of alleles in Reticulitermes aculabialis populations of Xi'an and Nanjing was 2.075 and 2.011, respectively; the average number of effective alleles (Ne) was 1.747 and 1.705, respectively; and the average observed heterozygosity (Ho) was 0.483 and 0.445, respectively. The expected mean value of heterozygosity (He) was 0.366 and 0.344, respectively, while the average value of the diversity index (I) was 0.566 and 0.534, respectively. All the above results indicated that the genetic diversity and the degree of genetic variation within Xi'an and Nanjing populations were similar, both at a moderate but relatively low level. Additionally, the genetic structure of the populations in Xi 'an and Nanjing was analysed by GenALEx software, showing that there was significant genetic differentiation (Fst=0.409) between the two populations, and the gene flow between the populations was relatively low (Nm = 0.375).

1 INTRODUCTION

Termites belong to Arthropoda: Insecta: Isoptera, which are the most primitive of the social insects. There are 2,935 species of termites in the world (Chand et al., 2018), belonging to 9 families and 283 genera. China has one of the highest abundances of termite species. The termite fauna of China is composed of 476 described species belonging to 44 genera in 4 families (Zhong and Liu, 2002).

At present, breeding termites causes a wide range of hazards, such as house destruction, dam breakage, ship sinking, bridge collapse, forest tree health damage and cultural relic damage; they directly interfere with people's normal life order (Rust and Su, 2012). The harm is usually isolated; thus, it does not draw much attention. Humans roughly understand the seriousness of the hazard but ignore its important relationship with environmental protection.

As a social insect, the distribution of genetic diversity at the population level is associated with the genetic structure at the colony level. However, due to its complex life history, it is difficult to analyse the genetic diversity and breeding system by collecting reproductive subterranean termites, and it is difficult to further study their biological characteristics and nest structure (Vargo, 2003).

With the development of molecular technology, microsatellite genetic markers have been used to detect the population genetic diversity and genetic differentiation of termites. The first microsatellite study of termites was by Vargo, who published 9 microsatellite markers and related primer sequences for R. flavipes (Vargo, 2000). In the same year, Vargo and Henderson published 12 microsatellite markers and their related primer sequences for Coptotermes formosanus (Vargo and Henderson, 2000).

Reticulitermes aculabialis, belonging to Rhinotermitidae, is a harmful termite in China and is distributed in 18 provinces and autonomous regions in China (Xing, Cui and Cheng, 1998). According to our investigation, Reticulitermes aculabialis is the main termite species that endanger garden plants in Shaanxi and Jiangsu. In recent years, the termite damage in the northwest region has become increasingly serious, and the damage in some areas is close to the south bank of the Yangtze River (Li et al., 2010).

Considering the significant impact of termite breeding hazards on the environment, this study intends to use the microsatellite primer marker SSR to investigate the genetic diversity of two different populations of Reticulitermes aculabialis. The results may serve as a support for the termite reproductive

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evolution mechanism and provide effective prevention and control strategies for environmental protection.

2 MATERIALS AND METHODS

2.1 Experimental Materials

Samples of Reticulitermes aculabialis were collected from 60 natural colonies in Xi'an and Nanjing, for a total of 240 experimental samples that were used as experimental materials. The experimental materials collected from the field were immediately immersed in absolute ethanol and stored at -20 ℃ until use (Table 1).

2.2 Experimental Instruments and Reagents

Table 1: Instruments and equipments used in the experiment.

Names of equipments	Models of equipments	Sources of experimental equipments
Water bath	HH-6	Guohua Electric Company
PCR amplification instrument	Mastercycler nexus gradient	Eppendorf, Germany
Ultra-clean workbench	AIRTECH	Suzhou Yida Boland Purification Laboratory System Equipment
Pressure steam sterilization pot	ZDX-35	Shanghai Shen'an Medical Instrument Factory
UVP gel imager	Bio-rad	Thermo Fisher Scientific
Vertical electrophoresis tank	DYCZ-24B	Beijing Liuyi Instrument Factory
Horizontal electrophoresis tank	DYCP-31DN	Beijing Liuyi Instrument Factory
Oscillator	Qilinbeier QL-901	Qilinbeier Instrument Manufacturing Co., Ltd.
Pure water system	Arium611	Sartorius, Germany
Electric constant temperature drying oven	TXQ-LQ-18SI	Shanghai Senxin Instrument Co., Ltd.
Decolorization shaker	Qilinbeier TS-1 orbital Shaker	Qilinbeier Instrument Manufacturing Co., Ltd.
Refrigerator	BCD-208k/A CJN	Qingdao Haier Co., Ltd.
Micropipette	Eppendorf	Eppendorf, Germany
Analytical Balances	METTLER TOLEDO	Switzerland
High speed refrigerated centrifuge	Eppendorf 5430R	Eppendorf, Germany
Vortex mixer	QL-901	Qilinbeier Instrument Manufacturing Co., Ltd.

Table 2: Names and Sources of experimental reagents.

Names of experimental reagents	Sources of experimental reagents
Blood genomic DNA Extraction Kit (centrifugal column type)	Shanghai Shenggong Biological Engineering Co., Ltd.
2×Taq PCR Mix	Xi'an Runde Biotechnology Co., Ltd.
ddH_2O	Xi'an Runde Biotechnology Co., Ltd.
600 bp DNA Marker I	Xi'an Runde Biotechnology Co., Ltd.
Microsatellite primer	Xi'an Runde Biotechnology Co., Ltd.
Goldview	Shanghai Shenggong Biological Engineering Co., Ltd.
Ethanol	Shanghai Shenggong Biological Engineering Co., Ltd.
TEMED	Shanghai Shenggong Biological Engineering Co., Ltd.

2.3 Sample Genome DNA Extraction

Genomic DNA was extracted using a DNA extraction kit (Table 2). The sample was detected by electrophoresis on a 10 g/L agarose gel. The concentration was measured by an ultraviolet spectrophotometer and stored at 4 °C (Table 1).

2.4 Microsatellite Primers, PCR Reaction Parameters and Genotype Products

Six pairs of microsatellite primers were selected; three pairs of primers, Ra132, Ra141 and Ra144, were selected by the laboratory and proved to have good polymorphism. The other three pairs of primers Rs03, Rs76 and Rs78 also proved to have good polymorphism (Vargo, 2000; Dronnet et al., 2004). The primer sequences and related parameters are shown in Table 3. The microsatellite primers were fluorescently labelled using semi-automatic fluorescent microsatellite markers, and the extracted whole genome DNA was amplified by PCR with the Mastercycler nexus GXS1 using synthetic fluorescent primers. The reaction solution was as follows: 7.5 μl of Mix (Table 2), 0.6 μl of forward and reverse primers, 3 μl of template DNA, and ddH2O to bring the total solution to 15 μl . The amplification procedure was as follows: 94°C pre-denaturation for 5 min, 30 cycles of 94°C denaturation for 30 s, suitable annealing temperature for 30 s, 72°C extension for 30 s, and a 72°C extension for 10 min.

A total of 3 μl of the amplified PCR product was taken for agarose gel electrophoresis, and high-quality PCR products were selected and sent to Shanghai Biotech for testing on an ABI3700 automatic analyser.

Table 3: Six pairs of microsatellite loci primers.

Primer	Primer sequences	Core repeat unit	The annealing temperature
Rs03	TCCTGACTGTACAAAGAAAAGTGG	(CT)9	58.2℃
	TGGCATCAAGCTACGTATTCA		
Rs76	AATCCGGGGAATTTCTTGAC	(AGTT)8	56.9℃
	CTGCATAACGATGTCTGCGT		
Rs78	GCTTCTCAAGAAGGACTGTGC	(AGTT)7	56.9℃
	GCCCCAGTTGAGATATGGAA		
Ra132	GATTGGTTTCCTCCGAATCA	(TTA)14	58.2℃
	AAAGACTACTGCCACCGGG		
Ra141	CACATTTGAGGTTCGCAAGA	(TTA)8	59.7℃
	GCCAGAAGGCCAATTACAGA		
Ra144	CAAATAGAGCTCCGTGTTTCG	(TTAG)7	56.9℃
	CCATAGAAACCTCCGAAAGG		

2.5 Statistical Analysis of Data

After data collection using GeneMarker 2.2.0 software, the length of the amplified product was read and calibrated for statistical analysis. The data format was converted using GenAlEx software based on an Excel macro calculation. GenAlEx6 software was used to transform the data format used by a different software package (Rod and Petere, 2006). The genetic

diversity and genetic structure index of each population, including percentage of diversity bands (PPL), number of observed alleles (Na), number of effective alleles (Ne) (Nei, 1973), observed heterozygosity (Ho), Nei's genetic diversity index (He) and Shannon information index (I) (Lewontin, 1995) for each pair of primers was calculated using Popgene32 software and Excel software. The polymorphic information content (PIC) of each microsatellite locus was calculated using Cervus software referenced by Smith (Jsc et al., 1997) and other methods. Application of GenAlEx 6.5 (Peakall et al., 2012) software was used to calculate genetic variation fixed index F-statistics (FIT, FIS, FST) (Wright, 1978) and gene flow between populations (Nm) (Whitlock and Mccauley, 2010).

3 RESULTS AND ANALYSIS

3.1 Analysis of SSR Genetic Diversity in Each Population of Reticulitermes Aculabialis

In this study, six pairs of SSR primers were used to analyse the genetic diversity of populations in Xi 'an and Nanjing. The calculation of genetic diversity parameters was performed using GenAlEx software (Table 3 and 4). The results showed that the polymorphic information content (PIC) ranged from 0.293 to 0.739, and the average polymorphic information content was 0.472. Of the six microsatellite loci, highly polymorphic seats with PIC values greater than 0.5 were observed in 2 of the 6 microsatellite loci. The PIC values of Rs 03, Rs 76, Ra 132 and Ra 141 were between 0.25 and 0.5, indicating that the six microsatellite loci used in this study showed moderately high polymorphism in the population of Reticulitermes aculabialis from Xi'an.
The number of alleles (Na) was 1.710 to 3, with an average of 2.075, and the number of effective alleles (Ne) was 1.431 to 2.441, with an average of 1.747, indicating that the alleles (Na) in this population were not evenly distributed and that the availability of alleles (Na) was low. The observed heterozygosity (Ho) values were 0.363 to 0.774, and the average observed heterozygosity was 0.483. The expected heterozygosity values (He) were 0.252 to 0.558, and the average expected heterozygosity was 0.366.

The larger the observed heterozygosity value, the greater the degree of genetic variation within the population; thus, the genetic variation within the population was at a moderately low level. The diversity index values (I) were 0.379 to 0.939, with an average value of 0.566, which was a medium but relatively low level, indicating that there was a certain diversity within the population, but the diversity was not high.

In the population of Reticulitermes aculabialis in Nanjing, the polymorphic information content (PIC) ranged from 0.260 to 0.721, and the average polymorphic information content was 0.483. Highly polymorphic seats at PIC values greater than 0.5 were observed in 2 of the 6 microsatellite loci, while Rs 03, Rs 76, Ra 132, Ra 141 had PIC values between 0.25 and 0.5, showing moderate polymorphism (Table 5). The six microsatellite loci used in this study also showed moderately high polymorphism in the population of Reticulitermes aculabialis from Nanjing. The number of alleles (Na) was 1.552 to 2.793, and the mean was 2.011. The number of effective alleles (Ne) was 1.326 to 2.337, and the mean was 1.705. The difference between the number of effective alleles (Ne) and the number of alleles (Na) was not very large, indicating that the alleles in the population are more evenly distributed. The observed heterozygosity values (Ho) were 0.190 to 0.638, the average observed heterozygosity was 0.445, the expected heterozygosity values (He) were 0.190 to 0.502, and the average expected heterozygosity was 0.344. Compared with the Xi'an population, the genetic variation between the two

populations was similar. The polymorphism index values (I) were 0.335 to 0.850, with an average value of 0.534, indicating a moderately low level of genetic variation and genetic diversity in the population.

3.2 Genetic Differentiation of Populations of Reticulitermes Aculabialis

GenAlEX software was used to calculate the total gene diversity index (FIT), the inbreeding coefficient (FIS) between individuals within the population and the genetic differentiation between populations Coefficient (FST) for 240 samples of termites in Xi'an and Nanjing. Additionally, analysis of the genetic structure of the termite population was conducted.

The results are shown in Table 6. Six microsatellite loci showed negative values in the total gene diversity range (FIT) of the population and negative values of the inbreeding coefficient (FIS), indicating that there was excess heterozygosity. The differentiation coefficient (FST) was 0.311~0.504; the maximum was detected at the Rs 03 site, and the minimum was detected at the Ra 141 site, with an average value of 0.409 (> 0.25). The gene flow (Nm)

was 0.246~0.553, with an average value of 0.375 (< 1), indicating that the gene flow between different geographical populations was obstructed, the level of gene exchange between populations was relatively low, and the genetic differentiation was relatively large.

Table 4: Genetic diversity of R. aculabialis in Xi'an.

Locus	Sample	Na	Ne	I	Ho	He	PIC
RS03	124	2.000	1.772	0.578	0.452	0.387	0.486
RS76	124	1.903	1.605	0.500	0.484	0.336	0.393
RS78	124	2.097	1.742	0.589	0.460	0.386	0.511
Ra132	124	1.742	1.489	0.409	0.363	0.274	0.410
Ra141	124	1.710	1.431	0.379	0.363	0.252	0.293
Ra144	124	3.000	2.441	0.939	0.774	0.558	0.739
Mean	124	2.075	1.747	0.566	0.483	0.366	0.472
SE	0	0.054	0.042	0.024	0.025	0.015	0.062

Table 5: Genetic diversity of R. aculabialis in Nanjing.

Locus	Sample	Na	Ne	I	Ho	He	PIC
RS03	116	1.552	1.326	0.289	0.190	0.190	0.496
RS76	116	2.172	1.760	0.606	0.534	0.390	0.496
RS78	116	2.034	1.656	0.537	0.414	0.346	0.553
Ra132	116	1.931	1.744	0.587	0.517	0.409	0.375
Ra141	116	1.586	1.409	0.335	0.379	0.228	0.260
Ra144	116	2.793	2.337	0.850	0.638	0.502	0.721
Mean	116	2.011	1.705	0.534	0.445	0.344	0.483
SE	0	0.058	0.044	0.027	0.026	0.017	0.064

Table 6: Results of F-statistics analysis and gene flow (Nm).

Locus	FIS	FIT	FST	Nm
RS03	-0.114	0.447	0.504	0.246
RS76	-0.404	0.125	0.377	0.413
RS78	-0.193	0.339	0.446	0.311
Ra132	-0.288	0.278	0.440	0.318
Ra141	-0.541	-0.061	0.311	0.553
Ra144	-0.333	0.172	0.379	0.409
Mean	-0.312	0.217	0.409	0.375
SE	0.062	0.073	0.028	0.044

4 DISCUSSION

4.1 Analysis of SSR Genetic Diversity in Two Populations of Reticulitermes Aculabialis

Analysis of the SSR genetic diversity of two populations in Xi'an and Nanjing showed the average number of alleles per SSR locus (*Na*=2.011~2.075), the average number of effective alleles (*Ne*= 1.705~1.747), the average observed heterozygosity (*Ho*=0.445~0.483) and the average expected heterozygosity (*He*=0.344~0.366), indicating that there was a certain degree of genetic diversity in both populations. As a very important indicator for measuring the genetic diversity of a population, the expected heterozygosity of microsatellites between 0.3 and 0.8 indicates that a population has higher genetic diversity (Nei and Takezaki, 1996). The average expected heterozygosity of *Reticulitermes aculabialis* in Xi'an and Nanjing was 0.355, which is greater than 0.3. The two loci of Rs 76 and Rs 78 used in this experiment were also involved in the genetic diversity of *R. grassei, R. santonensis* and *R. flavipes*. The expected heterozygosity of the two loci was 0.38~0.73 and 0.46~0.85, respectively, which are both higher than the expected heterozygosity of Rs 76 and Rs 78 of *Reticulitermes aculabialis* in Xi'an and Nanjing. Therefore, this result indicates that the genetic diversity of *Reticulitermes aculabialis* in Xi'an and Nanjing is generally rich, which has a positive influence on the environment. This may be due to inbreeding pressures, genetic mutations, small populations or human factors.

4.2 Analysis of the Genetic Structure of Reticulitermes Aculabialis Populations

The key indicator reflecting the genetic differentiation between populations is the genetic differentiation coefficient (*Fst*). In general, a large range of continuously distributed populations will lead to a gradual decrease in gene flow as a result of increased geographical distance leading to genetic differentiation (Barton, 2001; Barton and Etheridge, 2010). Wright (1978) proposed that when $0 < Fst < 0.05$, the genetic differentiation of the population is non-existent. When $0.05 < Fst < 0.15$, the population has moderate genetic differentiation (Wright, 1978) . When $0.15 < Fst < 0.25$, the population has high genetic differentiation. In this study, the genetic differentiation coefficient (*Fst*) was 0.311~0.504, and the average value was 0.409, indicating that the genetic differentiation between the two populations was great. Compared to the Chinese honeybee population (*Fst*=0.002~0.037) in Qinba Mountain, the genetic differentiation between Xi'an and Nanjing is higher (Wang, et al., 2004) .

The extent of gene flow (Nm) is an important factor affecting genetic differentiation among populations. Inter-gene communication can reduce the degree of differentiation by increasing the genetic variation between populations (Wright, 1974). Therefore, when the gene flow (Nm) is greater than one, the genetic differentiation caused by genetic drift

and selection can be effectively inhibited, and eventually the population tends to become consistent (Whitlock, 1999). However, when the gene flow (Nm) between populations is less than one, genetic differentiation may occur due to the obstruction of gene flow among sub-populations (Chen, et al., 2004).

In this experiment, the gene flow (Nm) of each microsatellite locus ranged from 0.226 to 0.553, with an average of 0.375, which revealed that the gene exchange between the two populations of Reticulitermes aculabialis in Xi'an and Nanjing was weak or non-existent, resulting in a higher genetic differentiation among populations.

The reason for the low gene exchange between the two populations was that the termites could not migrate over a wide range due to the short and limited flight time and poor flight ability, which limited the genetic communication among the populations. Additionally, urban areas were densely populated, and human activities were frequent. Inter-city eco-tourism and urbanization construction led to environmental fragmentation, leading to isolated nesting and breeding of termites, which in turn affected genetic communication among populations.

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