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Population Diversity of *Odontotermes formosanus* (Shiraki) (Termitidae, Macrotermitinae) from Different Geographic Locations in Anhui Province, China

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Abstract

Genetic differentiation, genetic exchange, and influence of natural geographic barrier on the genetic structure of 20 geo-populations of *Odontotermes formosanus* sampled from different regions in Anhui province, China were detected using ISSR. Seventy-nine polymorphic loci were detected with nine ISSR primers, and the percentage of polymorphic bands was 87.78%. The average number of alleles per locus was 1.8778 ± 0.3294, and the effective number of alleles was 1.4741 ± 0.3438. The *Nei's* gene diversity and Shannon information index were 0.2832 ± 0.1696 and 0.4307 ± 0.2274, respectively. All the populations were divided into two groups through UPGMA clustering analysis based on *Nei's* genetic distance. One group comprised geo-populations A, C, and J, and the other group consisted of the remaining clusters. Mantel test results revealed no significant correlation between genetic similarity and geographical distance, as well as between elevation. High levels of genetic diversity, genetic mutation, and genetic differentiation were also detected among the geo-populations of *O. formosanus*. This study revealed the gene flow and possible migration paths of *O. formosanus*, which are necessary for continuous monitoring and prevention of this species.

Introduction

Odontotermes formosanus (Termitidae, Macrotermitinae), a soil-dwelling termite, is mainly distributed in the Southern provinces along the Yellow and Yangtze Rivers in China (Mühlemann et al., 1995). Although *O. formosanus* promotes balance in the agroecosystem and biomass degradation in forest systems (Black & Okwakol, 1997), this species is an important pest which can damage many garden trees, plantations and dikes with high economic losses (Huang et al., 2000; Huang et al., 2006; Ge et al., 2008; Appel et al., 2012). As such a most destructive pest, *O. formosanus* are usually controlled and treated in urban areas by chemical pesticide in time (Huang et al., 2006).

An abundant geographic division and significantly different climatic conditions, topography, and vegetation exist in Anhui province as a result of the diversity in terrain and geomorphology, as well as the fact that the Yangtze River and the Huai River both traverse Anhui Province from west to east (https://en.wikipedia.org/wiki/Anhui#Geography). Because of the influence of natural and anthropogenic factors, *O. formosanus* is capable of long-distance migration and short-range dispersal (Hu et al., 2007). Geographical location, environmental situation, and host pressure may lead to genetic differentiation and changes in the genetic structure of insects in different geographical populations (Yang et al., 2015). The relationship between genetic similarity and the distances of geographic locations has been pointed out when focused on

individual intra- and interspecific populations in east China (Long et al., 2009). Obviously, study on the genetic diversity or similarity through methods of molecular biology and genetics to determine whether there is gene exchange between different populations of one species seems very important. Also, through those studies, the possible insect-source site and migration path could be well pointed out, which will be great helpful for controlling of this pest.

Molecular genetic markers are useful tools for distinguishing colony distinctness, inferring colony breeding structure, tracking colonies after insecticide treatment and determining whether termites were exterminated entirelly (Vargo, 2003a). Many researchers obtained results about microsatellite markers on some termites, such as *Macrotermes michaelseni* (Kaib et al., 2000), *Cubitermes subarquatus* (Harry et al., 2001), *Mastotermes darwiniensis* (Goodisman et al., 2001), *Coptotermes lacteus* (Thompson et al., 2000), *Coptotermes formosanus* (Vargo & Henderson 2000; Vargo et al., 2003), *Reticulitermes flavipes* (Vargo, 2000, 2003b), *Reticulitermes chinensis* (Huang et al., 2013) and *Reticulitermes speratus* (Hayashi et al., 2002). Such studies shed light on the genetic studies on termites. While as a kind of representative fungus-growing termites, most current literatures about *Odontotermes* contain the studies of the evolution and transmission of its symbiotic fungal (Aanen et al., 2002 and 2009; Osiemo et al., 2010; Yashiro et al., 2011), the cellulases of symbiotic bacterium (Su et al., 2016; Duan et al., 2017), the biology and ecology of this insect (Huang et al., 2008; Appel et al., 2012). Husseneder (2013) surveyed the population genetic of *O. formosanus* sampled from some provinces of south China through microsatellites techonology which showed that populations tested were genetically differentiated and the gene flow was appreciable with long geographical distance. But considering the effect of the special geographic condition, such as some mountains and rivers, as mentioned in last paragraph, more genetic studies on *O. formosanus* in Anhui province need to be done.

Inter-simple sequence repeat (ISSR) is more reliable and easily used for studies on genetic diversity than other molecular markers based on PCR technology, such as random amplified polymorphic DNA (RAPD), amplification fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) (Nybom, 2004; Long et al., 2009). In this system, PCR is performed on a short DNA sequence between two simple sequence repeat (SSR) sequences in genomic DNA with a single oligomerized nucleotide primer containing anchored 1-4 bases in the 3′ or 5′ end of the sequence, which are widely available and exhibits fast evolutionary rate (Esselman et al., 1999; Tanya et al., 2011; Hu et al., 2015). In addition, the features of long sequence and high annealing temperature ensure that ISSR-PCR would exhibit high stability and reproducibility (Nagaoka & Ogihara, 1997; Tsumura et al., 1996; Qian et al., 2001; Naik et al., 2017). Although ISSR is widely used in studies on various plants (Qian et al., 2001; Cao et al., 2006; Tanya et al., 2011), fungi (Menzies et al., 2003) and some insects (Li et al., 2009; Dutta et al., 2012; Barbosa et al., 2014), but it was less used on the study of termites (Long et al., 2009).

So in this study, the relationship between genetic and geographical distance, as well as genetic similarity and elevation, were investigated among geographical populations of *O. formosanus* sampled from different regions in Anhui province of China.

Materials and methods

Sample collection and identification

Thirty natural populations were sampled from different sites with typical geographical features in Anhui Province, China from June 2011 to October 2011. Twenty populations were identified as *O. formosanus*. All of the samples were stored in anhydrous ethanol and stored at −20 °C. Collected data are shown in Table 1 and Fig 1.

Samples were first identified using morphological features and then through molecular identification by amplifying the mitochondrial DNA COII gene (Sperling et al., 1994; Wallman & Donnellan, 2001; Beckenbach et al., 1993). The total DNA was extracted from the head of a soldier termite to avoid contamination from termite intestinal symbiotic microbes. Genomic DNA was extracted following previously described methods (Long et al., 2009). DNA precipitate was dissolved in 20 µl of 1×TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). DNA quality and quantity were determined using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the concentration was regulated at 20 ng μl^{-1}. The DNA samples were then stored at −20 ºC prior to ISSR–PCR analysis.The primers C2F2 (Lo et al., 2004) and BtLys (Liu & Beckenbach, 1992) were used to amplify a partial sequence of the COII gene through PCR on a C1000 Touch™ thermal cycler (Bio-Rad Hercules, CA, USA).

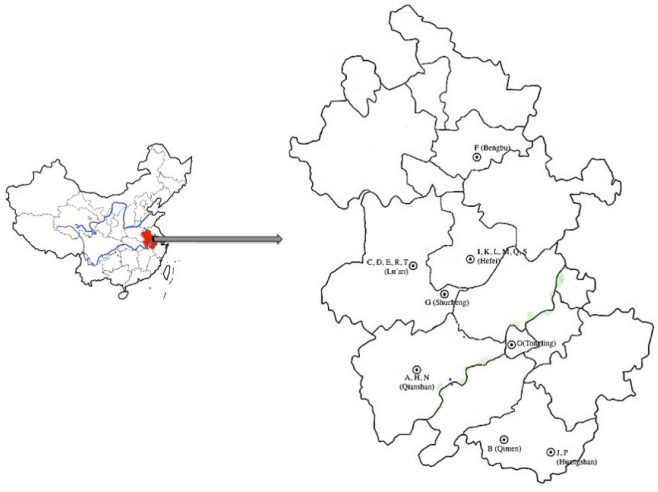

Fig 1. Locations where *O. formosanus* populations were sampled in China. The explanation of A-T can be found in Table 1. The green shadow represented the Yangtze River.

Table 1. Collection data of geo-populations of *O. formosanus*.

Geo-population	Sampling site	Geographical coordinates	Elevation (m)	Collection date
A	Qianshan	116°33′02.63″ E 30°39′39.97″ N	50	2010.09
B	Qimen	117°42′55.44″ E 29°51′05.89″ N	117	2010.09
C	Lu'an	116°32′48.36″ E 31°45′32.70″ N	66	2010.07
D	Lu'an	116°30′14.28″ E 31°45′42.96″ N	42	2010.07
E	Lu'an	116°29′38.70″ E 31°45′24.54″ N	67	2010.07
F	Bengbu	117°11′08.49″ E 32°44′54.61″ N	103	2010.08
G	Shucheng	117°01′21.83″ E 31°19′25.71″ N	92	2010.07
H	Qianshan	116°33′04.89″ E 30°39′39.97″ N	53	2010.09
I	Hefei	117°10′27.86″ E 31°51′04.76″ N	45	2010.07
J	Huangshan	118°08′43.20″ E 30°05′05.58″ N	576	2010.09
K	Hefei	117°09′41.04″ E 31°50′41.90″ N	66	2010.07
L	Hefei	117°15′14.82″ E 31°51′42.31″ N	32	2010.07
M	Hefei	117°15′57.75″ E 31°51′27.83″ N	33	2010.07
N	Qianshan	116°33′06.72″ E 30°39′40.88″ N	54	2010.09
O	Tongling	117°47′59.10″ E 30°56′50.73″ N	9	2010.08
P	Huangshan	118°08′48.72″ E 30°10′58.68″ N	522	2010.09
Q	Hefei	117°09′40.04″ E 31°50′39.90″ N	67	2010.07
R	Lu'an	116°32′46.80″ E 31°45′37.26″ N	67.2	2010.07
S	Hefei	117°10′11.35″ E 31°51′03.94″ N	64	2010.07
T	Lu'an	116°29′40.08″ E 31°45′21.48″ N	69.1	2010.07

PCR was carried out in a 20 µl reaction mixture containing the following components: 13.8 µl of sterile deionized water, 2.0 µl of 10× PCR buffer, 1.2 µl of 25 mM Mg^{2+}, 0.4 µl of 10 mM dNTPs, 0.3 µl of 10 µM Primer1, 0.3 µl of 10 µM Primer2, 0.5 µl of 5 U μl^{-1} rTaq DNA polymerase, and 1.5 µl of 20 ng μl^{-1} templates. The following thermal profile was used for PCR amplification: initial denaturation for 3 min at 94 ºC; 29 cycles of denaturation for 30 s at 94 ºC, annealing for 30 s at 55 ºC, and extension for 1 min at 72 ºC; and final extension for 10 min at 72 ºC. PCR products were sent to Shenggong Inc. (Shanghai, China) for purification and sequencing.

ISSR amplification and product detection

According to 96 ISSR primers released by Columbia University in Canada (UBC set 9) and former research results (Long et al., 2009), nine primers, which can generate clear, bright, and multifarious fragments, were used to provide polymorphic markers for detection of genetic diversity (Table 3). PCR was carried out in a 20 µl reaction mixture containing the following components: 13.2 µl of sterile deionized water, 2.0 µl of 10× PCR buffer, 2.4 µl of 25 mM Mg^{2+}, 0.3 µl of 10 mM dNTPs, 0.6 µl of 10 µM primers, 0.5 µl of 5 U μl^{-1} r*Taq* DNA polymerase, and 1.0 µl of 20 ng μl^{-1} template. Amplification

Table 3. Inter-simple sequence repeat (ISSR) primers and corresponding information.

Primer name	Core sequence (5'–3')	Attached bases	Annealing temperature (ºC)	Number of amplified bands	Number of polymorphic bands	PPB † (%)	H ‡	I §
IS01	$(AC)_8$	T	55	10	9	90	0.3009±0.2041	0.4461±0.2687
IS07	$(ATG)_6$		53	10	9	90	0.3333±0.1683	0.4917±0.2341
IS09	$(TG)_6$	CC	50	10	8	80	0.2804±0.2069	0.4153±0.2839
IS13	$(CA)_6$	AG	50	11	9	81.82	0.3050±0.2039	0.4482±0.2751
IS14	-$(GA)_5$	CAA-	46	10	9	90	0.2941±0.1956	0.4418±0.2525
IS16	$(CAC)_4$	RC	50	8	7	87.50	0.3105±0.1809	0.4611±0.2534
IS18	$(GA)_8$	YT	48	11	9	81.82	0.1953±0.1695	0.3149±0.2344
IS23	$(GAG)_4$	RC	50	10	10	100	0.3267±0.2007	0.4805±0.2582
IS24	$(GAA)_6$		50	10	9	90	0.3207±0.1904	0.4735±0.2525
Total				90	79	87.78		

† Percentage of polymorphism bands
‡ Nei's (1973) gene diversity
§ Shannon's Information index [Lewontin (1972)]

was performed on the PCR apparatus (C1000 thermal cycle, Bio-Rad) under the following cycle profiles: initial denaturation for 5 min at 94 °C; 39 cycles of denaturation for 45 s at 94 °C, annealing for 45 s at 46.0 °C to 55.0 °C (depending on primers used, Table 3), and extension for 1.5 min at 72 °C; and final extension for 10 min at 72 °C. The amplification products were resolved electrophoretically on 1.0% agarose gels at 180 V in 1× TAE buffer (2 M Tris, 1 M acetic acid, and 100 mM EDTA, pH 8.0) for 1 h. The products were then stained with SYBR® Green and photographed under the GelDoc-It® imaging system (UVP, LLC, USA).

Data analysis

The nucleic acid sequences of the COII gene of all samples were sorted, edited, and subjected to BLAST search using BIOEDIT 7.0.5.3 (Hall, 1999) and submitted to GenBank to select geo-populations of *O. formosanus*. All the ISSR amplification bands were read using the Gel-Pro Analyzer software (Media Cybernetics, Silver Spring, MD, USA). The bands, no matter strong or weak, were scored as present (1) or absent (0) for each DNA sample to form a two-dimensional matrix. Population genetic parameters were statistically analyzed using two assumptions: 1) the populations were in Hardy–Weinberg equilibrium and 2) the bands with the same electrophoretic mobility were considered as the amplification products of identical DNA fragments in the genome (Lu et al., 2009). The number of polymorphic loci, percentage of polymorphic bands (PPB), *Nei's* gene diversity (H), Shannon's information index (I), genetic distance, and genetic similarity were calculated using POPGEN32 v 1.31 (Yeh et al., 1997; Zhao et al., 2009).

To represenet the relationship among populations, a UPGMA dendrogram was generated using *Nei's* unbiased genetic distance with the NTSYSpc v 2.1 software package (Rohlf, 2000). A clustering map was generated through a tree plot module. The geographical distance of 20 *O. formosanus* populations were obtained using Distance Calculator (www.daftlogic.com/projects-google-maps-distance-calculator.htm) to analyze the existence of isolation by distance among populations from different locations (Table 2), and elevation difference was also calculated using the altitude of collecting sites. The correlation of the symmetric matrix constituted by genetic and geographical distance, as well as genetic similarity and elevations, was further compared and analyzed using MXCOMP programs and mantal test in NTSYSpc-2.1.

Table 2. Geographical distance among all populations. (unit: m)

Populations	A/H/N	B	C/R	D	E/T	F	G	I/K/L M/Q/S	J/P	O
A/H/N	0									
B	139357	0								
C/R	124204	240274	0							
D	125760	241508	1603	0						
E/T	135127	269331	60110	60596	0					
F	265635	342305	154643	153155	197820	0				
G	83845	213251	46609	48187	56865	200527	0			
I/K/L/M/Q/S	146078	223962	68809	68333	128914	122830	99615	0		
J/P	197413	61886	286005	287061	321862	367616	265044	257440	0	
O	122652	121722	152763	153440	201658	223071	148062	112271	145957	0

Results

ISSR profile

Nine ISSR primers generated 90 bands, which correspond to an average of 10 bands per primer. Of these bands, 87.78% were polymorphic among the 20 populations. Each band in the same size presented an unique ISSR genotype, which indicated an extensive genetic variation among the collected populations. The electrophoregram of amplification products using the primer IS16 was shown in Fig 2, and information on all ISSR primers and the corresponding amplification are shown in Table 3. All primers generated multiple distinct bands. IS16 and IS09 generated least amounts of polymorphic bands, namely, 7 and 8 bands,

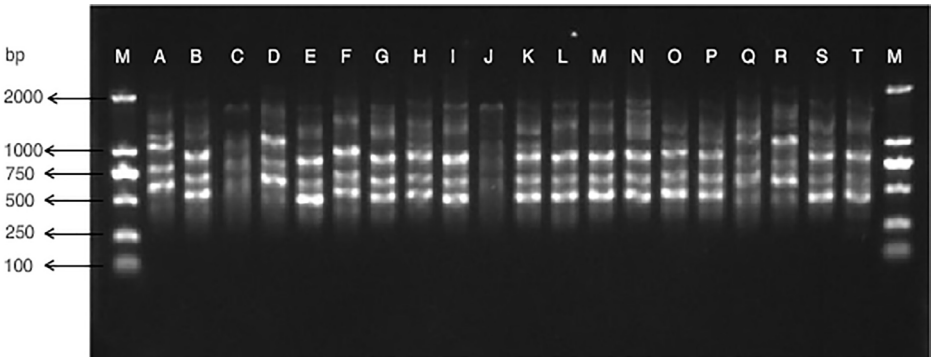

Fig 2. Amplification products of the primer IS16 for analyzed populations (A to T). M in the lane 1 represent DL2000 marker which produce 5 different sized bands (2000bp, 1000bp, 750bp, 500bp, 250bp, 100bp).

respectively, whereas IS23 generated the highest amount of polymorphic bands, namely, 10 bands. Overall, 90 bands were generated with nine primers and the PPB was 87.78%.

Analysis of genetic diversity

According to the genetic similarity and genetic distance of *O. formosanus* geo-populations, the average number of alleles (*Na*) per locus was 1.8778 ± 0.3294 and the effective number of alleles (*Ne*) was 1.4741 ± 0.3438. The Nei's gene diversity (*H*) and Shannon information index (*I*) were 0.2832 ± 0.1696 and 0.4307 ± 0.2274, respectively, and the total gene diversity was 0.2832 ± 0.0288. These data revealed high genetic diversity at the population level among all samples and the abundant intraspecific variation in *O. formosanus*. By analyzing genetic similarity among the populations (Table 4), we obtained 231 correlation coefficients ranging from 0.4333 to 0.9222 with an average of 0.6828, which indicated a high range of genetic variation among the populations studied in this research. The highest level of genetic similarity (0.9222) was observed between geo-populations K and L, as well as geo-populations C and J, which indicated that these populations were genetically related and shared the same genetic components.

Relationship between population genetic differentiation and geographical location

The relationships of two sets of data (between genetic distance and geographic distance, between genetic identity and difference in elevation) are shown in Figs. 3 and 4. Data points were scattered without a discernible pattern. Mantel test results revealed no significant correlation between genetic and geographical distance (r = 0.10517, p = 0.8062), as well as between genetic similarity and elevation (r = −0.12512, p = 0.0707). This finding indicated no significant isolation by distance. Thus, proximity of sample collection did not affect genetic similarity.

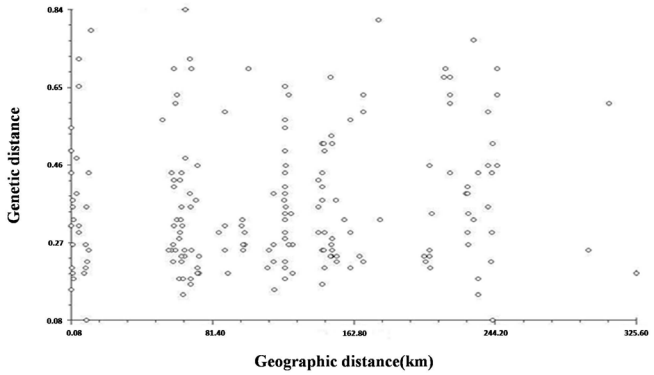

Fig 3. Correlation between genetic and geographical distance. The data points were generated by MXCOMP programs and shown as a scattered pattern. No significant correlation were identified between genetic and sample geographical distance by Mantel test (r = 0.10517, p = 0.8062).

Table 4. Genetic similarity coefficient (above diagonal) and genetic distance (below diagonal) among overall populations based on ISSR markers.

Population	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*	*P*	*Q*	*R*	*S*	*T*
A	****	0.6556	0.8333	0.6778	0.5778	0.6333	0.5556	0.6444	0.6111	0.8000	0.6000	0.5889	0.6000	0.5778	0.6111	0.5667	0.6889	0.6667	0.6000	0.5667
B	0.4223	****	0.5556	0.6444	0.7444	0.8222	0.7222	0.7444	0.7111	0.5667	0.7444	0.6667	0.6778	0.7000	0.6889	0.7778	0.6778	0.7000	0.7667	0.8000
C	0.1823	0.5878	****	0.6222	0.5222	0.5333	0.4333	0.5667	0.5333	0.9222	0.5000	0.4889	0.5000	0.5222	0.5111	0.4667	0.6556	0.6111	0.5444	0.4889
D	0.3889	0.4394	0.4745	****	0.7000	0.7111	0.7000	0.6333	0.7333	0.6333	0.6556	0.6889	0.6333	0.7000	0.6889	0.6444	0.7444	0.6778	0.7222	0.6889
E	0.5486	0.2951	0.6497	0.3567	****	0.7667	0.8444	0.7556	0.7889	0.5333	0.8000	0.8111	0.8222	0.8000	0.7889	0.8333	0.6444	0.7333	0.8667	0.8556
F	0.4568	0.1957	0.6286	0.3409	0.2657	****	0.7222	0.7000	0.7778	0.5444	0.7444	0.7333	0.7222	0.7000	0.7111	0.7778	0.7667	0.7667	0.7667	0.8222
G	0.5878	0.3254	0.8362	0.3567	0.1691	0.3254	****	0.7333	0.7667	0.4444	0.7778	0.8333	0.7556	0.7778	0.7444	0.7889	0.6444	0.6222	0.8000	0.8333
H	0.4394	0.2951	0.5680	0.4568	0.2803	0.3567	0.3102	****	0.8111	0.5556	0.8444	0.7889	0.7556	0.7333	0.7444	0.7444	0.6444	0.7111	0.7778	0.7222
I	0.4925	0.3409	0.6286	0.3102	0.2371	0.2513	0.2657	0.2094	****	0.5444	0.8333	0.8222	0.7667	0.7444	0.8000	0.7778	0.7222	0.7222	0.8111	0.7778
J	0.2231	0.5680	0.0810	0.4568	0.6286	0.6080	0.8109	0.5878	0.6080	****	0.5111	0.5000	0.5111	0.5333	0.5000	0.4556	0.6444	0.6000	0.5333	0.5000
K	0.5108	0.2951	0.6931	0.4223	0.2231	0.2951	0.2513	0.1691	0.1823	0.6712	****	0.9222	0.7778	0.7778	0.7667	0.7889	0.6444	0.6667	0.8222	0.7889
L	0.5295	0.4055	0.7156	0.3727	0.2094	0.3102	0.1823	0.2371	0.1957	0.6931	0.0810	****	0.7667	0.7667	0.7778	0.8000	0.7000	0.6778	0.8111	0.8222
M	0.5108	0.3889	0.6931	0.4568	0.1957	0.3254	0.2803	0.2803	0.2657	0.6712	0.2513	0.2657	****	0.7778	0.8111	0.7889	0.6444	0.7778	0.8000	0.7889
N	0.5486	0.3567	0.6497	0.3567	0.2231	0.3567	0.2513	0.3102	0.2951	0.6286	0.2513	0.2657	0.2513	****	0.7222	0.8111	0.6667	0.6444	0.7778	0.8111
O	0.4925	0.3727	0.6712	0.3727	0.2371	0.3409	0.2951	0.2951	0.2231	0.6931	0.2657	0.2513	0.2094	0.3254	****	0.8222	0.6778	0.7889	0.8556	0.8000
P	0.5680	0.2513	0.7621	0.4394	0.1823	0.2513	0.2371	0.2951	0.2513	0.7862	0.2371	0.2231	0.2371	0.2094	0.1957	****	0.6333	0.7222	0.8111	0.8667
Q	0.3727	0.3889	0.4223	0.2951	0.4394	0.2657	0.4394	0.4394	0.3254	0.4394	0.4394	0.3567	0.4394	0.4055	0.3889	0.4568	****	0.7333	0.6889	0.7000
R	0.4055	0.3567	0.4925	0.3889	0.3102	0.2657	0.4745	0.3409	0.3254	0.5108	0.4055	0.3889	0.2513	0.4394	0.2371	0.3254	0.3102	****	0.7778	0.7444
S	0.5108	0.2657	0.6080	0.3254	0.1431	0.2657	0.2231	0.2513	0.2094	0.6286	0.1957	0.2094	0.2231	0.2513	0.1560	0.2094	0.3727	0.2513	****	0.8333
T	0.5680	0.2231	0.7156	0.3727	0.1560	0.1957	0.1823	0.3254	0.2513	0.6931	0.2371	0.1957	0.2371	0.2094	0.2231	0.1431	0.3567	0.2951	0.1823	****

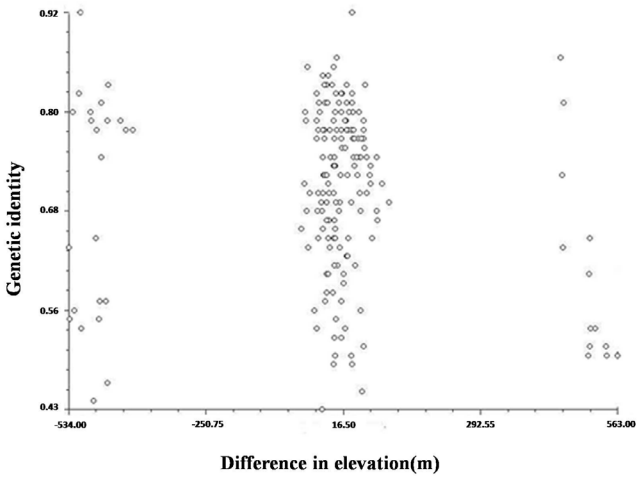

Fig 4. Correlation between genetic identity and difference in elevation. The data points were generated by MXCOMP programs and shown as a scattered pattern. No significant correlation were identified between genetic similarity and sample elevation by Mantel test ($r = -0.12512$, $p = 0.0707$).

UPGMA cluster analysis based on Nei's genetic distance

A 0/1 matrix was constructed for all ISSR amplification bands by using the Gel-Pro Analyzer software, and a dendrogram was generated with the NTSYSpc-2.1 software (Fig 5). Twenty geo-populations sampled from Anhui province were separated into two groups if 0.68 (genetic similarity coefficient) was set as a boundary. Group I included geo-populations C, J and A. Geo-populations A and C were distributed near and were both located in the Northwest of Anhui Province, whereas geo-population J clustered into this group despite being sampled from Huangshan and separated from geo-populations A and C by a natural geographic barrier (i.e., the Yangtze River). Within a single clade, which is divided into at least two groups, twelve geo-populations (E, S, P, T, O, M, G, N, K, L, I and H) from the central part of Anhui Province formed one sub-group in Group II. Similar to geo-populations C and J, geo-populations B and F gathered into another sub-group despite being separated by the Yangtze

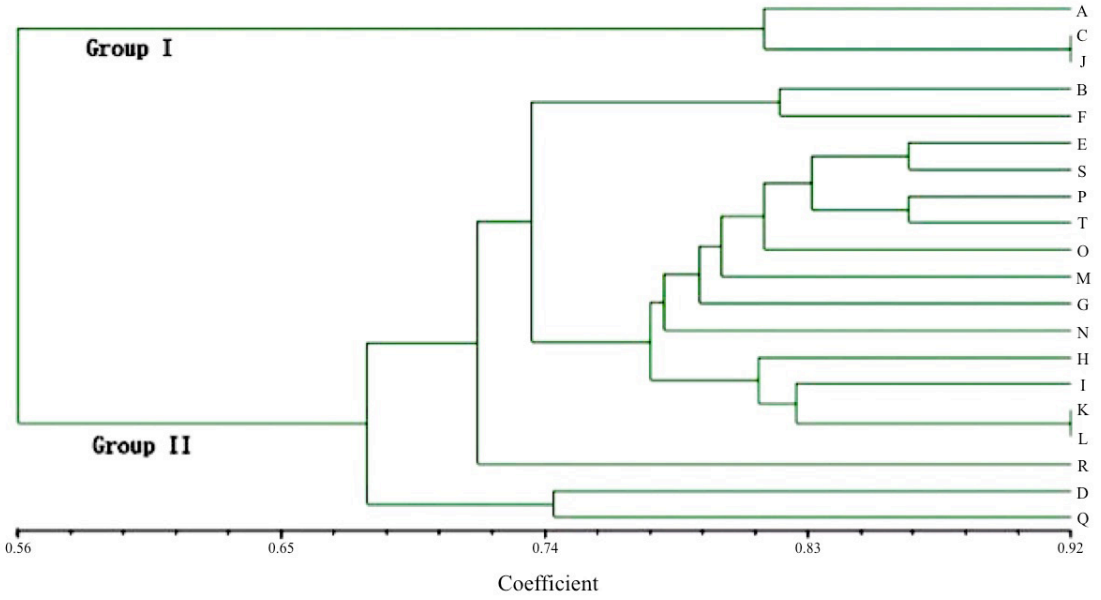

Fig 5. Dendrogram after unweighted pair-group method with arithmetic mean of *O. formosanus* based on Nei's genetic distance. Population codes are explained in Table 1.

River (geo-population B was sampled from Qimen and F from Bengbu). These two clusters gathered with geo-population R and then combined with another cluster, which was generated by geo-populations D and Q.

Discussion

As Botstein et al. (1980) proposed the range of polymorphic loci by PPB, all the nine ISSR primers presented high polymorphism and are potentially effective markers for the analysis of genetic diversity and phylogenetic relationship of geo-populations. In this study, only primer IS23 can get as high as 100% PPB. But all the same primers except for IS16 generated 100% PPB in another study on termites (Long et al., 2009). The difference should be related to the species of termites. The objects of this study were all *O. formosanus*, while inter-species termites from *Odontotermes*, *Reticultitermes* and *Coptotermes* were analyzed with high polymorphic loci.

Twenty populations investigated here are distributed in those natural areas, including Jianghuai hilly area, Dabie mountain area, plains along the Yangtze River, and mountainous areas of South Anhui, which generally cover the whole Anhui Province. The relationship between genetic variation and the geographical environment is important in research on genetic diversity and genetic distance, which represent the extent of genetic differentiation between two populations. The pattern of dendrogram was consistent with the result calculated by POPGEN32, but not the geographical structure entirely. For example, the result that the nearest

differentiation distance between geo-population C and J or between K and L were consistent with the result of genetic similarity (0.9222). In fact, geo-population C (from Lu'an) and J (from Huangshan) are separated by the Yangtze River and the Yellow Mountain with 286km long distance, while geo-population K and L are sampled from the same city. The genetic structures of geo-populations B, F, P, and T were the same condition (Table 2, Fig 1). The lowest level of genetic similarity was observed between geo-populations C and G, which indicated that their genetic components varied greatly despite that the distance between them was only about 46km. These results showed no significant correlation between genetic similarity and geographical distance. And the genetic diversity of populations also depends on the breeding structure and the origin of the nest (Husseneder et al., 2012). This phenomenon was also similar with the study done by Husseneder (2013). He pointed out that populations from different provinces (Guangdong, Jiangxi, and Hubei) were genetically differentiated while the genetic distance between populations was surprisingly small. Huang (2013) found that the Huanggang population was separated from other populations indicated by the largest genetic distance, while the populations of Changsha, Chongqing-1 and Chongqing-2 shared smaller genetic distance although they were far enough in the actual geography. The long-range dispersal by alates and/or transport by human perhaps were the reason. So here, we also considered that this may be due to artificial factors that gave rise to one population as the offspring of the other population, or similar gene selective pressures, such as host, terrain features, geomorphology, and climatic factors.

Whether it was due to the similar gene selective pressure among populations or other artificial factors, further experiment need to carry out, such as the confirmation of the presence of gene exchange and gene flow among the populations of *O. formosanus* in the central part of Anhui Province. Frequent communicating activities or inborn ability of short-range dispersal of the species might be the possible reason. Long-distance migration of *O. formosanus* should also be noted for prevention and control. However, this study only analyzed *O. formosanus* in Anhui Province; thus, research on population genetic relationships, genetic structure, and migration pattern in a wider range must be performed.

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