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The Analysis of Genetic Diversity of *Periplaneta americana*

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ABSTRACT

We analyzed the genetic diversity on *Periplaneta americana* of 12 populations consisting of 360 individuals with 16 high polymorphic markers. The results showed that the number of alleles per locus ranged from 16 to 32, with an average value 24.5. The observed heterozygosity value (HObs) varied from 0.481 to 0.906 and the mean HObs was 0.698; the expected heterozygosity value (HExp) varied from 0.625 to 0.928 and the mean HExp was 0.840. The polymorphic information content (PIC) varied between 0.602 and 0.923. The analysis of Genepop and Popgene showed that a relatively high level of genetic diversity was revealed: the observed number of alleles was 10.0781; the effective number of alleles was 5.2746. The observed heterozygosity was 0.6882, and the expected heterozygosity was 0.7791, Nei's expected heterozygosity was 0.7661, Shannon's information index was 1.8056. Most loci were found to be deviated from HW test. A higher level of genetic differentiation was detected among populations with mean Fst was 0.0891, the mean Nm was 2.5573, the genetic identity ranged from 0.4224 to 0.9405, the genetic distance ranged from 0.0613 to 0.8617. UPGMA cluster analysis indicated that the 12 populations were grouped into three major clusters, which showed that there had rich genetic diversity in *P. americana*, and the genetic differentiation to some extent, the differentiation in certain degree may result from geographical isolation and barriers to gene flow. These results would provide the basis of further study on genetic diversity and structure.

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Authors' Contribution
LFZ and XYZ collected the samples. XYZ and BSY analysed the data. LFZ and BSY conducted the experiments and analysed the results. All authors discussed the results and wrote the manuscript.

Key words
***Periplaneta Americana*, Microsatellite Markers, Polymorphism, Genetic diversity.**

INTRODUCTION

Cockroaches, which are among the first neopteran insects to appear in the fossil record, are extremely generalized in most morphological features. They are usually omnivorous and are included in the sub-order Blattodea that together with Mantodea (mantids) form the Order Dictyoptera (Tamaki *et al*., 2014). *Periplaneta americana* is commonly named American cockroach which belongs to Order Blattodea Family Blattidae, Genus *Periplaneta*. As a large insect family in insect groups, *P. americana* has the strongest and oldest vitality and most successful reproduction in the world, *P. americana* has strong viability, although perennial live in the insanitary circumstance such as sewer, it can survivel well in such dirty conditions, which means that it has eubacteria. It has a long history in Shen Nong's Herbal Classic with the effects of its anti-inflammatory, analgesic and tissues repairing. The recent research showed that *P. americana* extract had the effect that can enhance human immunity, antitumor. In recent years, people make huge researches on the *P. americana* medical effects, and develop many medicines, such as Liver dragon capsule, Kang Fuxin, Xin-Mai-Long and etc as well as*P. americana* edible dried worm power and extracting refined powder. Now in China, people start widely artificial breeding on *P. americana*, some areas built up standardized artificial breeding system. *P. americana* breeding becomes an industrialization stream. Artificial breeding population generation is too many or breeding population genetic diversity is low, which will lead to provenance quality decline as well as the reproductive capacity and efficient medicine. To ensure the provenance quality and great provenance breeding, we need to make a research on the *P. americana* germplasm resources and make test and value on the provenance heredity, which can give the theory supporting on the future provenance introduction and germplasm breeding. Microsatellites were the first widespread PCR-based marker (Tautz, 1989; Weber and May, 1989). They have since become the most used molecular marker for population genetic analysis, having been discovered to occur frequently in the genomes of organisms from nearly every taxonomic group (Estoup and Angers, 1998). Microsatellite is the DNA associate repeat sequences that formed on 1-6 nucleotides basic repeating unit, also called simple sequence repeats (SSRs) or short tandem repeats (STRs). Microsatellite widely exists on eukaryote, prokaryote and viral gene group, and divided into three types: unitary type (also called pure); compound type and interrupted type. Simple sequence repeats provides a powerful tool for determining genetic

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variation in insects genetic diversity for their relative abundance, co-dominant inheritance, multiple alleles (Powell *et al*., 1996). Due to these merits, microsatellite is widely used on the molecular genetic markers, as well as the best discrimination rate on the population genetic structure study and the strongest revealing DNA mark. On the insect study, microsatellite is widely used on the insect heredity diversity, group heredity structure analysis, behavior and habit study, genetic map construction specific gene location and the system's evolution and occurrence.

Genetic diversity is an important index to measure the level of population genetic difference. It reflects the ability of a species to adapt to the environment and its potential to be reformed and utilized. Selections of genetically diverse parental lines based on morphological and quality traits are often difficult because of the high degree of genetic identity of breeding variety (Mishra *et al*., 2014). This is because of the narrow genetic origin of commercially cultivated varieties as well as the self fertile nature of the species (Lashermes *et al*., 1996). The generations of new and improved breeding variety can be facilitated by incorporating new sources of genetic diversity from diverse germplasm sources (Mishra *et al*., 2014). Analyses on genetic diversity and relationship among the species of *P. americana* could also provide useful information for the conservation of genetic resources and the establishment of a *P. americana* breeding program (Aichiyousfi *et al*., 2016). Genetic diversity could give a general guide make suitable cross combinations for the selection of valuable traits with large possible applications in agriculture, food industry and medicine (Aichiyousfi *et al*., 2016). Microsatellite markers are widely used in genetic diversity research. Assessment of genetic diversity is an important component of insect breeding programs. Genetic assessment of germplasm helps in identifying parents with different agronomic traits for effective recombination in hybridization program (Mishra *et al*., 2014).

This study used sixteen microsatellite locations to analysis genetic diversity among twelve *P. americana* geographical populations. The study filled the *P. americana* microsatellite marks screening vacancy, established the foundation study for the following *P. americana* germplasm resource and genetic relationship identification, population genetic diversity and group genetic construction etc aspects. It also provided important basic data for breeding high quality provenances in artificial culture. Analyses on genetic diversity and relationship among the geographical populations of *P. americana* could also provide useful information for the conservation of genetic resources and the establishment of *P. americana* breeding program (Aichiyousfi *et al*., 2016).

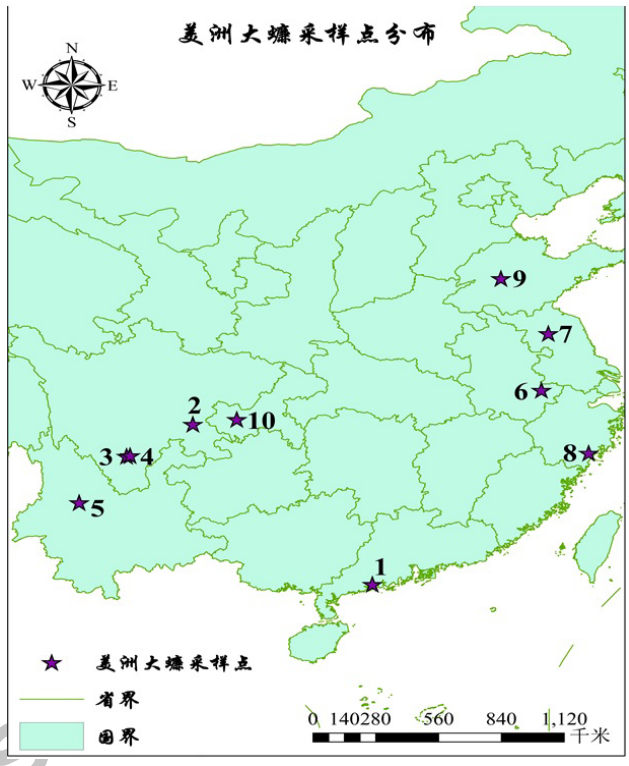

Fig. 1. Sampling sites: 1, Yangjiang county, Guangdong province (GDYJ); 2, Rong county, Zigong city, Sichuan province (ZGRX); 3, Xichang city, Liangshan prefecture, Sichuan province (LSXC); 4, Lizhou county, Liangshan prefecture, Sichuan province (LSLZ); 5, Dali city, Yunnan province (YNDL); 6, Xuancheng city, Anhui province (AHXC); 7, Huai'an city, Jiangsu province (JSHA); 8, Wenzhou city, Zhejiang province (ZJWZ); 9, Tai'an City, Shandong province (SDTA); 10, Chongqing Municipality (ZGCQ).

MATERIALS AND METHODS

Sample collection

Collection sites used in our study are shown in Figure 1. All the individuals were collected from ten different locations in China and stored in the -80 degree refrigerator

Genomic DNA extraction

Genomic DNA was extracted from the legs of each individual using blood/cell/tissue genomic DNA extraction kit (TIANGEN biochemical technology (Beijing) Co. Ltd. Each population selected 30 individuals, and DNA concentration was estimated by standard spectrophotometric methods at 260 and 280 nm UV lengths by Thermo Scientific Nanodrop 2000 and the integrity by gel electrophoresis in a 1.5% agarose gel. The resuspended

DNA was then diluted in sterile distilled water to 10 ng/μL concentration for use in amplification reactions. DNA was marked and preserved at -20°C in refrigerator.

Population amplification

In our obtained experiment, we screened 16 pairs of microsatellite sites with polymorphism, and they are shown in Table I. No one showed a departure from Hardy–Weinberg equilibrium, and no one were found to be related with disequilibrium. These loci will facilitate future ecological and population genetic studies of *P. americana*. According to the polymerase chain reaction (PCR) procedures outlined by Booth *et al* (2007), respectively in the 12 geographic populations for PCR amplification. SSR PCR amplifications were performed in 25 μL reactions containing 1.5 μL genomic DNA templates, 10×PCR buffer (Mg^{2+}) 2.5μL, 2.5 mmol/L dNTP 1.0 μL, 0.5 μL primers (F-Primer, R-Primer), and 5 U/μL Taq DNA polymerase 0.3 μL, ddH_2O 18.7 μL. Amplification reactions were performed on a PCR Thermal Cycler under the following conditions: initial denaturation at 95°C for 4 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C to 62°C for 40 s, extension at 72°C for 30 s and a final 10 min elongation step at 72°C, preserved at 4°C. The PCR products were analyzed electrophoretically on 1.5% agarose gels in 1 × TAE Buffer at 110 V for 30 min. A total of 1 μL 6 × loading buffer was added to each reaction before electrophoresis.

Table I.- The screening information of polymorphism microsatellite.

No. Primers	Primer sequences(5'-3')	Repeat copy	Fluorescence	Tm(°C)	Length (bp)
Pam5	TTCCAACACGCCCTACTGAA TGCATGCATACTGTACATGGA	$(ATGT)_{11}$	FAM	56	235
Pam7	AACAAACAAAATCTGCACCTGA ACCTTTCACTCTGTACAGCTCT	$(ACTA)_{11}$	HEX	55	276
Pam16	AGGGTTGTTCAAAAGTCACTAGA TAGAGAAGGGGTGGGAGTGA	$(GTAT)_{11}$	FAM	58	204
Pam17	TCCACGTGTAATGAGCCCAA TCCCCATCGTGTAACCTGTG	$(ATTT)_{11}$	HEX	57	242
Pam22	ACTACTTGGAACTGGTCTCCA CACTTGCATACATATTGCACACA	$(ATGT)_{11}$	FAM	57	241
Pam28	CCACGACCCACTACAGCATA CGTGCAAGTTCATCGTGTTG	$(TATG)_{11}$	HEX	57	250
Pam30	TGCGTGCGTATAGGATGGAT GCGCACCCAACTTTTGAAAT	$(TATT)_{11}$	FAM	58	206
Pam32	CTCCATCTAGTGTGCCTCGA TGCACTTTCCTTAACGCTCT	$(CATT)_{11}$	HEX	54	232
Pam35	TGCCATGGGAGAAAGAACAAC TGCTCTCTCTCTCTTTCCCTG	$(TGAA)_{11}$	HEX	55	222
Pam44	AAAATTGGACTGCGGCAAGT CCTCCTGGTGTATTCTGTGC	$(CATT)_{11}$	FAM	53	246
Pam54	AACGGTTAGTCGACTCGGTT GAAAGCTCCTTGGGCAGAAC	$(CATT)_{11}$	HEX	57	239
Pam82	CTTTGGACTGGGAGCTCTCA AGAGAAAGCAGGAGTCGACC	$(TGAT)_{11}$	FAM	57	249
Pam83	GATCCTCAAGAGACTCCGGG TTACGCCGAAAACCACACTG	$(TTAT)_{11}$	FAM	58	244
Pam89	CGAGTAACAACGGACGCCTA TCTCACATAGCGGACAGTCG	$(TTCT)_{11}$	FAM	56	248
Pam91	ACATTTACCCAAAACTGTGCAG TGCGAAGGATCTCGTGTACA	$(GAAT)_{11}$	HEX	56	230
Pam94	ACAACCACTCTCATATCGCCA AGCAACTCTGGATCGGTAGG	$(TGTA)_{11}$	HEX	55	247

Microsatellite genotyping

PCR products conform to the stripe size, mixed FAM and HEX marking samples, with tinfoil to keep in dark place, being sent Tsingke (Chengdu) Biological Technology Co. Ltd. for genotyping, used Genescan and Genotyper software for analyzing the scanned results.

Data analysis

Samples were analyzed hierarchically to take into consideration the aims outlined above. Summary population statistics (Allele, A), (observed heterozygosity, HObs), (expected heterozygosity, HExp), (polymorphism information content, PIC) were calculated using the Cervus 3.0 (Marshall *et al*., 1998).

Departures from Hardy-Weinberg equilibrium (HWE) and genotypic linkage equilibrium were tested for each sample across all loci in GENEPOP, version 4.0 (Raymond and Rousset, 1995; Rousset, 2008). Bonferroni correction for multiple tests was applied to each of these tests.

With the POPGENE 1.32 (Yeh *et al*., 1999) program, estimates were made of the allelic frequency for each locus, the effective allele number, the percentage of polymorphic loci and Shannon's index as a measure of genetic diversity and genetic distance and identity (Nei, 1972, 1978). Average genetic diversity or HExp was calculated based on the formula proposed by Nei (1973).

To observe the relations between the studied population and individuals of *P. americana* graphically populations, dendrograms were done by using UPGMA, based on Nei's (1972) genetic distances.

RESULTS

Polymorphic information

The number of alleles, PIC, HObs and HExp of 16 microsatellite loci in the 360 individuals for 12 geographical populations were analyzed. The number of allele obtained for 16 microsatellite loci varied from 16 to 32, with a total of 392, with 24.5 at each locus. The range of HObs was between 0.481 and 0.906, and the average was 0.698. The HExp varied from 0.625 to 0.928, and the mean was 0.840. The null allele frequency was negative at Pam5, -0.0599. The remaining 15 loci ranged from +0.0006 to +0.2770. The PIC varied between 0.602 to 0.923, with an average of 0.824, which was highly polymorphic (Table II).

Genetic diversity

Hardy-Weinberg equilibrium (HWE) test

The results of HWE test showed that Pam5 was out of equilibrium in all populations, and the other sites were in the range of 8.33% to 91.67% (Table III).

Table II.- The polymorphism parameters of 16 microsatellite markers.

Locus	k	N	HObs	HExp	PIC	HWE	F(Null)
Pam5	24	360	0.906	0.821	0.799	ND	-0.0599
Pam7	16	360	0.753	0.846	0.827	*	+0.0548
Pam16	21	360	0.856	0.859	0.844	NS	+0.0006
Pam17	37	360	0.669	0.893	0.882	***	+0.1426
Pam22	25	360	0.489	0.871	0.858	***	+0.2770
Pam28	27	360	0.653	0.871	0.858	***	+0.1470
Pam30	22	360	0.589	0.625	0.602	***	+0.0105
Pam32	18	360	0.725	0.894	0.883	***	+0.1041
Pam44	27	360	0.481	0.791	0.765	***	+0.2583
Pam35	29	360	0.686	0.928	0.923	ND	+0.1522
Pam82	22	360	0.828	0.907	0.898	***	+0.0441
Pam54	18	360	0.717	0.740	0.699	NS	+0.0152
Pam83	24	360	0.581	0.774	0.759	***	+0.1444
Pam91	22	360	0.750	0.841	0.825	***	+0.0493
Pam89	32	360	0.761	0.900	0.892	***	+0.0808
Pam94	28	360	0.722	0.878	0.866	***	+0.0974
Mean	24.5	360	0.698	0.840	0.824		

Note: HExp, expected heterozygosity; PIC, polymorphic information content; HObs, observed heterozygosity; HWE, Hardy-Weinberg equilibrium test; K, number of alleles per locus; N, number of individuals; F(Null), null allele frequency estimate. Significance of Hardy-Weinberg tests (Raymond and Rousset 1995): *=P < 0.05, **=P <0.01, ***=P < 0.001; NS, not significant; ND, not done.

Table III.- HW equilibrium test for 16 microsatellite loci in 12 populations.

Locus	Population											
	XCYS	AHXC	ZGRX	LSLZ	LSXC	GDYJ	JSHA	YNDL	SDTA	ZJWZ	ZGCQ	XCYZ
Pam5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Pam7	0.0498	0.0080	0.0947	0.0029	0.7971	0.3883	0.1693	0.0128	0.4629	0.2934	0.2061	0.6640
Pam16	0.5794	0.3298	0.0317	0.3664	0.2086	0.0407	0.7360	0.2492	0.0000	0.1114	0.5789	0.1266
Pam17	0.0000	0.0566	0.0000	0.0000	0.0031	0.0169	0.0155	0.0143	0.8930	0.1634	0.2052	0.0000
Pam22	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0027	0.0006	0.2216
Pam28	0.2608	0.0468	0.1667	0.0000	0.4132	0.0520	0.2630	0.2979	0.0232	0.0955	0.0000	0.0000
Pam30	0.0668	0.0054	0.7248	0.2944	0.8379	0.2183	0.1078	0.3235	0.4319	0.1112	0.0000	0.0000
Pam32	0.0051	0.0122	0.1235	0.0024	0.0026	0.0080	0.1634	0.0190	0.4815	0.0053	0.2696	0.1153
Pam44	0.0000	0.0000	0.0000	0.0142	0.0988	0.0043	0.0000	0.0003	0.3736	0.0000	No info	0.1815
Pam35	0.0114	0.0043	0.0025	0.1726	0.0045	0.2550	0.5184	0.0000	0.3664	0.0000	1.0000	0.0012
Pam82	0.0270	0.4620	0.2992	0.1790	0.1578	0.0099	0.1357	0.0092	0.7049	0.0942	0.7053	0.1059
Pam54	0.6284	0.3012	0.5836	0.3924	0.1849	0.3796	0.2198	0.2131	0.0759	0.3724	0.5937	0.0009
Pam83	0.2697	0.0110	0.4530	0.0060	0.5721	0.0000	0.0000	0.0000	0.0000	0.0148	0.0240	0.0000
Pam91	0.0063	0.0310	0.0669	0.0039	0.0013	0.0215	0.0809	0.0000	0.0000	0.0000	0.0000	0.0000
Pam89	0.0164	0.2108	0.0575	0.3557	0.3361	0.0000	0.1116	0.0655	0.0003	0.4093	0.5401	0.0946
Pam94	0.3357	0.0000	0.2693	0.3835	0.9045	0.0036	0.0041	0.0036	0.8696	0.0262	0.3920	0.0560

Note: No info, no information.For details of population, see Figure 1.

Table IV.- Summary of genetic variability for 12 populations.

Pop	Obs Het	Exp Het	Nei	na	ne	I	P (%)	LD
XCYS	0.7229	0.8341	0.8202	11.7500	6.5969	2.0339	100	98
AHXC	0.6917	0.7904	0.7772	10.4375	5.3928	1.8673	100	69
ZGRX	0.7146	0.8032	0.7899	10.9375	5.5033	1.9094	100	80
LSLZ	0.7000	0.8077	0.7942	11.0000	5.8001	1.9252	100	79
LSXC	0.7333	0.7969	0.7836	10.6875	5.2788	1.8702	100	75
GDYJ	0.6000	0.8610	0.8467	11.6250	6.3493	2.0258	100	78
JSHA	0.7229	0.8150	0.8015	10.3125	5.7383	1.8954	100	71
YNDL	0.6333	0.7766	0.7636	10.1250	4.8248	1.7928	100	56
SDTA	0.7396	0.6651	0.6540	6.2500	3.3198	1.3200	100	41
ZJWZ	0.6979	0.7508	0.7383	9.2500	4.5244	1.6970	100	50
ZGCQ	0.6250	0.6240	0.6136	6.5000	3.7907	1.3203	100	11
XCYZ	0.6771	0.8241	0.8103	12.0625	6.1755	2.0094	100	162
Mean	0.6882	0.7791	0.7661	10.0781	5.2746	1.8056		72.5

Note: Expected homozygosty (HExp) and heterozygosity (HObs) were computed using Levene (1949). Nei's (1973) expected heterozygosity; na, observed number of alleles; **ne, ...?**; I, Shannon's Information index [Lewontin (1972)]; P%, the percentage of polymorphic loci; LD, linkage disequilibria. Pop, population. For details of population, see Figure 1.

Population diversity analysis

By Popgene 1.31, the population diversity information is shown in Table IV. The HObs ranged from 0.6000 to 0.7396 in 12 geographic populations, the lowest in GDYJ population was 0.6000, the highest in SDTA was 0.7396, the average HObs was 0.6882; The HExp ranged from 0.6240 to 0.8610, the lowest was ZGCQ population (0.6240), the highest was GDYJ population (0.8610), and the average HExp was 0.7791. It could be seen that there was no bound relationship between HObs and HExp. Nei's HExp ranged from 0.6136 to 0.8467, the lowest was Chongqing population (0.6136), the highest was GDYJ population (0.8467), and the average Nei's was 0.7661. It could be seen that the HExp was consistent with the size of Nei's HExp.

The number of observed alleles ranged from 6.5000 to 12.0625, the average observed alleles was 10.0781, the highest was XCYZ population (12.0625), the lowest was ZGCQ population (6.5000); and the number of effective alleles was between 3.3198 and 6.5969. The average

number of effective alleles was 5.2746, the lowest of SDTA population was 3.3198, and the highest of XCYS population was 6.5969. It could be seen that the XCYS population had the highest values of observed alleles and effective alleles.

Table V.- The homozygosty and heterozygosity for 16 microsatellite loci.

Locus	Obs Hom	Obs Het	Exp Hom	Exp Het	Nei
Pam5	0.0944	0.9056	0.1789	0.8211	0.8200
Pam7	0.2472	0.7528	0.1544	0.8456	0.8445
Pam16	0.1444	0.8556	0.1407	0.8593	0.8581
Pam17	0.3306	0.6694	0.1070	0.8930	0.8918
Pam22	0.5111	0.4889	0.1292	0.8708	0.8696
Pam28	0.3472	0.6528	0.1285	0.8715	0.8703
Pam30	0.4111	0.5889	0.3749	0.6251	0.6242
Pam32	0.2750	0.7250	0.1059	0.8941	0.8929
Pam44	0.5194	0.4806	0.2090	0.7910	0.7899
Pam35	0.3139	0.6861	0.0718	0.9282	0.9270
Pam82	0.1722	0.8278	0.0930	0.9070	0.9058
Pam54	0.2833	0.7167	0.2600	0.7400	0.7389
Pam83	0.4194	0.5806	0.2265	0.7735	0.7724
Pam91	0.2500	0.7500	0.1585	0.8415	0.8403
Pam89	0.2389	0.7611	0.0995	0.9005	0.8992
Pam94	0.2778	0.7222	0.1219	0.8781	0.8769
Mean	0.3023	0.6977	0.1600	0.8400	0.8389

Note: Expected homozygosty and heterozygosity were computed using Levene (1949). Nei's (1973) expected heterozygosity.

Shannon's information index could be used to estimate the genetic differentiation within the population. The greater Shannon index was, the greater the genetic diversity was, and the higher the degree of population differentiation was. The Shannon's information index of all populations ranged from 1.3200 to 2.0339, with a mean of 1.8056, the lowest was SDTA population (1.3200), and the highest was XCYS population (2.0339), the number of effective alleles was consistent with the information index. The percentage of polymorphic loci can reflect the size of population genetic diversity, and the frequency of polymorphic loci equal the ratio of polymorphic loci divide total loci. The percentage of polymorphic loci at population level was 100 percent and they had high polymorphism. There were some differences between Shannon's information index and population polymorphic locus rate, which indicated that the Shannon's information index and polymorphic locus rate had different results in explaining the genetic variation of the population, and it could be seen that the genetic diversity of the XCYS population was relatively rich. The linkage disequilibrium was between 11 and 162, with an average of 72.5 per population.

Table V shows the variation range of the observed homozygosity of the 16 microsatellite loci was from 0.0944 to 0.5194, the average was 0.3023, the lowest was the locus Pam5 and the highest was the locus Pam44; the variation range of the HExp was from 0.4806 to 0.9056, the average was 0.6977, the lowest was the locus Pam44, and the highest was the locus Pam5. It could be seen that the observed homozygosity is opposite to the HObs.

The range of expected homozygosity was from 0.0718 to 0.3749, the average was 0.1600.The lowest locus was Pam 35, and the highest locus was Pam30. The HExp ranged from 0.6251 to 0.9282, with an average of 0.8400, the lowest was locus Pam30, and the highest was locus Pam35; The range of Nei's HExp was from 0.6242 to 0.9270, with an average value of 0.8389. the lowest was Pam 30, and the highest was Pam35. It could be seen that the expected homozygosity was on contrary to the HExp, and the HExp was the same as the Nei's HExp.

Table VI.- Summary of F-statistics and gene flow for 16 microsatellite loci.

Locus	Fis	Fit	Fst	Nm
Pam5	-0.1445	-0.1044	0.0351	6.8773
Pam7	0.0519	0.1086	0.0598	3.9323
Pam16	-0.0629	0.0030	0.0620	3.7834
Pam17	0.1930	0.2493	0.0697	3.3356
Pam22	0.3720	0.4378	0.1048	2.1344
Pam28	0.1667	0.2499	0.0998	2.2540
Pam30	0.0179	0.0566	0.0394	6.0979
Pam32	0.1397	0.1880	0.0562	4.1976
Pam44	0.2701	0.3916	0.1665	1.2517
Pam35	0.1387	0.2598	0.1406	1.5280
Pam82	0.0227	0.0861	0.0649	3.6046
Pam54	-0.0182	0.0301	0.0475	5.0158
Pam83	0.1649	0.2484	0.1000	2.2511
Pam91	-0.0427	0.1075	0.1441	1.4853
Pam89	0.0556	0.1536	0.1038	2.1586
Pam94	0.0697	0.1764	0.1146	1.9307
Mean	0.0869	0.1682	0.0891	2.5573

Note: Nm, Gene flow estimated from Fst = 0.25(1 - Fst)/Fst. The relative excess of homozygotes or heterozygotes compared with panmictic expectations relative to all populations (Fit), within populations (Fis), and among populations (Fst).

Population genetic differentiation analysis

F-statistics (Fis, Fit and Fst) were used to analyze genetic structure in populations (Nei, 1987). These measures represent relative excess of homozygotes or heterozygotes compared with panmictic expectations relative to all populations (Fit), within populations

(Fis), and among populations (Fst) (Shuyskaya *et al*., 2017). One estimate of Nm (the number of migrants per generation) was estimated using the extent of genetic differentiation among populations: Nm = 0.25(1−Fst)/Fst (Nei, 1987). The genetic differentiation of the population was examined by the total inbreeding coefficient (Fis), inbreeding coefficient within the population (Fit) and population differentiation coefficient (Fst) of each locus. High value of Fst indicates high genetic differentiation of populations. The results are presented in Table VI. The Fis of Pam5, Pam16, Pam54, Pam 91 were -0.1445, -0.0629, -0.0182, -0.0427, and the other loci ranged from 0.0179 to 0.3720. The average Fis of all loci was 0.0647. The Fit was from Pam5 (-0.0427) to Pam22 (0.4378) and the average Fit was 0.1682. Both Fis and Fit were negative, indicating that there was no inbreeding in the population. When Fis was negative and Fit was positive, indicating that there was inbreeding within the subpopulation. Fst was from 0.0351 to 0.1665, Pam44 significantly contributed to the result. The lowest locus was Pam5. The Fst average was 0.0891. According to Fst, the genetic differentiation of 91.09% of *P. americana* population existed in geographical population, and the genetic variation among geographical population was about 8.91%. On the population level, the range of gene flow (Nm) ranged from Pam5 (1.2517) to Pam44 (6.8773), with an average Nm of 2.5573.

The genetic relationship on different geographical populations could be analyzed according to Nei's genetic identity (I) and genetic distance (D). It could be seen from Table VII that the genetic identity of the 12 populations ranged from 0.4224 to 0.9405. The genetic identity between XCYS population and ZGRX population was the highest (0.9405), and the genetic identity between SDTA population and YNDL population was the lowest (0.4224). The I ≥ 0.8 among the populations of XCYS, AHXC, ZGRX, LSLZ, LSXC, GDYJ, JSHA, YNDL, XCYZ, which indicated that there were frequent gene exchange among these geographic populations. The genetic distance of all populations ranged from 0.0613 to 0.8617. The closest genetic distance was ZGRX population and XCYS population, and the furthest was SDTA and YNDL populations.

Population cluster analysis

The UPGMA clustering algorithm from SSR analysis grouped the 12 *P. americana* populations into three major clusters (Fig. 2). Among the three major clusters, the first one comprised of four minor clusters. The first minor cluster included a total of four *P. americana* populations, they were GDYJ, YNDL, LSXC, XCYZ population respectively. The second minor cluster included a total of three *P. americana* populations, they were XCYS, ZGRX, LSLZ population. The third minor cluster included JSHA population. The fourth minor cluster included AHXC population. The second one comprised of two minor clusters, they were SDTA, ZJWZ population respectively. The second one comprised of two minor clusters, they were SDTA, ZJWZ population, respectively. The third one represented by a single population, it is ZGCQ population. The samples collected from same province clustered together which could be attributed to the substantial gene flow between adjacent population and the influence of geographical origin on genetic diversity. The study demonstrated the existence of substantial genetic variation in *P. americana* which could be utilized in *P. americana* germplasm conservation and improvement program.

Table VII.- Nei's genetic identity (above diagonal) and genetic distance (below diagonal) for 12 populations.

Pop	XCYS	AHXC	ZGRX	LSLZ	LSXC	GDYJ	JSHA	YNDL	SDTA	ZJWZ	ZGCQ	XCYZ
XCYS	****	0.7497	0.9405	0.9256	0.9079	0.7728	0.8783	0.8234	0.5040	0.6454	0.5690	0.9075
AHXC	0.2881	****	0.7567	0.7160	0.7125	0.6416	0.8150	0.6408	0.5724	0.7295	0.5258	0.7666
ZGRX	0.0613	0.2788	****	0.9335	0.8820	0.7922	0.8834	0.8246	0.4517	0.6078	0.5383	0.9050
LSLZ	0.0773	0.3341	0.0688	****	0.8840	0.7720	0.8734	0.8415	0.4892	0.5983	0.6250	0.9186
LSXC	0.0966	0.3390	0.1256	0.1232	****	0.8119	0.8575	0.8467	0.5035	0.6332	0.5129	0.9114
GDYJ	0.2577	0.4437	0.2329	0.2588	0.2084	****	0.7911	0.7976	0.4412	0.5594	0.4275	0.8034
JSHA	0.1298	0.2045	0.1240	0.1353	0.1538	0.2344	****	0.8825	0.5448	0.6879	0.6118	0.8987
YNDL	0.1943	0.4450	0.1929	0.1726	0.1664	0.2262	0.1250	****	0.4224	0.5405	0.5211	0.8390
SDTA	0.6851	0.5579	0.7948	0.7150	0.6862	0.8183	0.6072	0.8617	****	0.6464	0.5471	0.4793
ZJWZ	0.4379	0.3153	0.4978	0.5136	0.4570	0.5809	0.3741	0.6152	0.4364	****	0.6186	0.6070
ZGCQ	0.5639	0.6428	0.6193	0.4700	0.6677	0.8498	0.4914	0.6518	0.6031	0.4803	****	0.5155
XCYZ	0.0971	0.2658	0.0998	0.0849	0.0928	0.2189	0.1068	0.1755	0.7353	0.4993	0.6625	****

For details of population, see Figure 1.

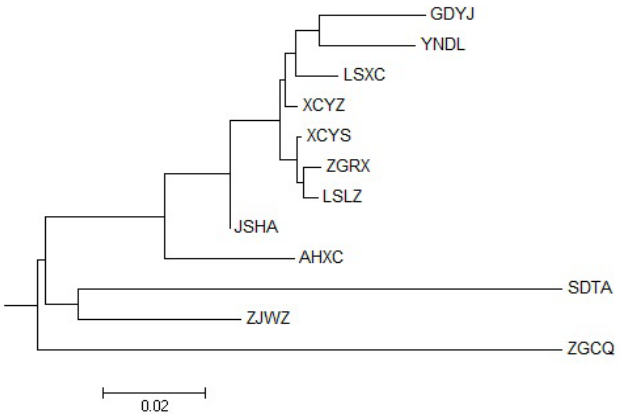

Fig. 2. UPGMA dendrogram based on Nei's genetic distance.

DISCUSSION

Genetic diversity of Periplaneta americana

The effect of polymorphic loci on genetic diversity

Genetic diversity can measure the degree of variation of genetic information, DNA is the main carrier of genetic information, so the diversity of DNA can directly reflect the degree of genetic variation of species. The genetic diversity of the population is mainly manifested in the number of alleles, heterozygosity and PIC. The PIC values, which were used as a reflection of allelelic diversity and frequency among the genotypes, varied from one locus to another (Mishra *et al*., 2014). The percentage of polymorphic loci is a good indicator of species genetic diversity. If P>50%, it is a highly polymorphic locus; if 25%<P≤50%, it is a moderate polymorphic locus; if P≤25%, it is a low polymorphic locus (Botstein *et al*, 1980). In general, the genetic diversity of broad-spread species is higher than that of narrowly distributed species. *P. americana* is widely distributed all over the world, and it is a widely distributed species.

The number of microsatellite markers used in genetic diversity studies is important for the accuracy and reliability of estimates of species' genetic heterozygosity, genetic distance, etc. According to the research (Nei, 1978), with over 50 loci, it is possible to obtain a good estimate of the average heterozygosity in the evaluated population, which compensates for a small number of individuals evaluated per population (Jaramillo-Ramirez *et al*., 2010). In this study, the percentage of polymorphic loci of 16 microsatellite loci was above 50%. These 16 microsatellite markers were highly polymorphic and could be used as effective genetic markers for the analysis of genetic diversity and phylogenetic relationship among the geographical populations of *P. americana*. The results also indicated that the population of *P. americana* had abundant genetic diversity.

Evaluation of genetic diversity by heterozygosity

Heterozygosity has expected heterozygosity (HExp) and observed heterozygosity (HObs). It refers to the probability that two alleles in the sample are different. The HExp is calculated according to the dominant allele frequency in the population. The results show that the number of effective alleles in microsatellite loci is proportion to the number of samples, but not to the HExp. The average proportion of heterozygotes within or on populations can be used to measure the degree of genetic differentiation among populations. The degree of population heterozygosity reflects the degree of genetic identity of population, which is inversely proportional to each other. The higher the degree of heterozygosity is, the lower the genetic identity of the population is, and the higher the genetic variation is, the higher the genetic diversity of the population is. Extreme values of heterozygosity also has been observed in other insect species such as the *Drosophila bifasciata* (HObs = 0.24) (Cariou *et al*., 1990), *Anopheles nuneztovari* (HObs = 0.34) (Posso *et al*., 2003), *Aedes aegypti* L. (HObs = 0.35) (Apostol *et al*., 1996) and the Australian wood cockroach *Panesthia australis* (HObs = 0.80) (Runciman *et al*., 2006).

The HExp of 12 geographical populations was 0.6240-0.8610 in the results of this study, and the average HObs was 0.6882. The HExp of the GDYJ and XCYS population was higher than other geographical locations, indicating that the genetic variation of the two geographic populations was higher and the genetic diversity was relatively rich. There were high levels of genetic variation within the 12 populations of *P. americana*, and abundant genetic diversity.

Effects of samples on genetic diversity

The collection of samples (including the quantity of samples, the geographical location of samples, the kinship between samples, etc.) is also crucial to the success of the experiment, and also the research results have key reference value and availability. An important basis for the representative of the samples and the reliability of the experimental results is that the number of samples collected is large enough, and the larger the sample is, the greater the number of alleles is, and the more abundant of the detected microsatellite markers. The study indicates that a small number of samples can be accepted if the genetic heterozygosity of large numbers of microsatellite markers is low and the genetic distance between populations is large (Nei, 1978). In random sampling, the reliability of the experiment is more than 95% when the number of samples

within varieties is more than 60, and when the subjects are large populations and the subgroups are included in large populations, each subgroup should be sampled, and the sampling number is more than 100. Samples capacity of a species should cover more than 25% of the species' number, and intra-species individuals should have no kinship within three generations and be able to represent the species in phenotypes, 25 males and 25 females (Barker, 1994). Twelve geographical populations of *P. americana* were collected in this study. Each population randomly selected 30 individuals for genetic diversity detection, which could roughly reflect the genetic diversity of each species.

In conjunction with the foregoing, genetic diversity could be maintained by a large and constant gene flow between population through natural dispersion (Reiter *et al*., 1995) and human-assisted migration via passive transport of individuals and oothecae (Chadee, 1990). Through either of these modes, the cockroach populations have the possibility of exchanging genetic material among them. This was evident when observing the number of effective migrants among the *P. americana* population evaluated. Besides, *P. americana* tends to move over short distances in the peridomiciliary and the movement from these areas towards the intradomiciliary is not quite clear (Schal and Hamilton, 1990). So, it can be supposed that the passive transport of individuals is one of the most important factors in the dispersion of individuals over great distances (Jaramillo-Ramirez *et al*., 2010). One of the possible reasons for the loss of genetic polymorphisms in halophytes may be their restricted ecological distribution (Wolff and Jefferies, 1987).

Genetic differentiation of Periplaneta americana

Fis is the number of inbred lines between individuals in subgroups and Fit is the number of inbred lines between subgroups within the total group, and their values range from 1 to 1. When the values of Fis and Fit are both positive, there is inbreeding within the population. Fst is a coefficient of genetic differentiation and an important index to reflect the degree of genetic differentiation among subpopulations. Fst of population can show the level of genetic differentiation between populations. If the value of Fst is between 0 and 0.05, the extent of genetic differentiation of the population is small; if the value of Fst is between 0.05 and 0.15, the extent of genetic differentiation is moderate; if the value of Fst is greater than 0.15, the extent of genetic differentiation is larger (Balloux *et al*., 2010). The result of this study showed that the Fis of Pam5 locus was -0.1445 and Fit was -0.1044, indicated that there was inbreeding within and outside the subgroup of this locus, while the Fst value was 0.0351, indicated that the locus Pam5 was slightly differentiated. The Fis of Pam16, Pam54, Pam91 was -0.0629, 0.0182, 0.0427separately, indicated that there was mild inbreeding among the three loci.

There are many reasons for the genetic differentiation of the population, such as somatic mutation, sexual reproduction, natural selection, gene flow, genetic drift and environmental effects. The research often uses the number of individuals migrated to measure the gene flow between different geographic populations. When Nem is less than 1.0, the gene flow is not sufficient to counteract the genetic differentiation caused by the independent evolution of the population. According to genetic drift theory, loss of rare alleles, increased differentiation and weakening of the isolation-by-distance component of genetic structure among populations can be expected (Barrett and Chalesworth, 1991; Ellstrand and Elam, 1993). Studies often use the migrated number of individuals to measure the gene flow between different geographic populations. The gene flow between populations was 2.5573 > 1, which indicated that the population of *P. americana* had the ability to resist a certain degree of population differentiation caused by genetic drift in the population.

Evolution of Periplaneta americana *population*

Genetic distance analysis can roughly estimate the evolutionary relationship on populations. Genetic variation on populations usually is expressed by the genetic distance calculated by allelic frequency. Genetic distance provides the best, effective and objective description of genetic variation among varieties. In this paper, genetic distance was calculated by Popgene 1.31 software and UPGMA cluster analysis was carried out. There are many formulas for calculating genetic distance. The most widely used genetic distance index is proposed in 1978 (Nei, 1978). According to the genetic distance and allelic frequency of Nei, UPGMA cluster analysis of population was constructed in this study (Fig. 2). The result displayed that 12 geographical populations were gathered into 3 groups, ZGCQ was one group, ZJWZ and SDTA were a group, AHXC and the remaining 8 groups was a group. In a group of 8 populations, GDYJ and YNDL were into a group, JSHA was a individual group, XCYS, ZGRX, LSLZ, XCYZ, LSXC were clustered, and the genetic distance of XCYS, ZGRX, LSLZ, XCYZ, LSXC was very small. DNA barcode technique was used to analyze genetic diversity. The difference of the three different regions was small, but the difference between populations was great. This result was similar to the present study.

The accuracy of the genetic distance calculation is related to the real geographical distance on populations, the number of microsatellite markers used, the polymorphism

of each microsatellite marker, the number of individuals sampled, and so on, and these factors are related to each other. Studies have shown that genetic distance calculation and phylogenetic tree construction are more reliable in detecting a large number of polymorphic microsatellite markers (Moazami-Guodarzi *et al*., 1997).

Significance of genetic diversity of Periplaneta americana

Genetic diversity is an important index to measure the level of population genetic difference. It reflects the ability of a species to adapt to the environment and its potential to be reformed and utilized. The indexes such as polymorphic loci percentage, Shannon information index and genetic diversity index were evaluated the population genetic diversity. Both Shannon information index and polymorphic locus rate can reflect the diversity, but the Shannon index can divide the variation of population into intra-population and inter-population variation, while the polymorphic locus rate can only reflect the size of population diversity. These two indicators reflect different depth of diversity. The percentage of polymorphic loci only reflects the surface phenomenon of diversity, while the Shannon index reflects some essential characteristics of species diversity to a certain extent. These indexes reflect the degree of genetic identity of the population: the lower the value is, the higher the degree of genetic identity and the lower the genetic diversity of the population is. Microsatellite markers are widely used in genetic diversity research. Assessment of genetic diversity is an important component of insect breeding programs. Genetic assessment of germplasm helps in identifying parents with different agronomic traits for effective recombination in hybridization program (Mishra *et al*., 2014), but population structure of *P. americana* has been poorly studied.

The study of the genetic diversity analysis of *P. americana* germplasm using SSR markers which targeted different geographic levels is poor. While similar studies have been done with other important cockroach species such as the urban pest *Blatella germanica* L. (Blattodea: Blaberidae) (Jaramillo-Ramirez *et al*., 2010). Researchers used enzymatic markers to study the genetic diversity of 31 of this species population originating from two French cities. It was observed both among and between the population of each of the cities was a significant genetic differentiation; however, a significant difference between cities was not found (Cloarec *et al*., 2010). Although there was evident substructuring at a local level, differentiation on a larger geographic scale could not be confirmed. These results oppose those found in this research with *P. americana*, which found a significant genetic difference between cities, besides substructuring at a local level (Jaramillo-Ramirez *et al*., 2010). Jaramillo-Ramirez *et al*. (2010) used the AFLP molecular marker technique to analyze the genetic structure of five *P. americana* (L.) populations from three cities (Cali, Popayán and Buenaventura) located in southwestern Colombia, the results showed that a high degree of subdivision within the *P. americana* population.

The number of effective alleles is an index to reflect the genetic variation of the population. The value of the effective alleles is closer to the absolute number of the detected alleles, which indicates that the distribution of the alleles in the population is more uniform. In this study, the average effective allele was 5.2746, the mean HExp was 0.7791, and the genetic diversity index between 0.6136 and 0.8467, the Shannon information index was over 1.32, with the highest value of 2.0339.

CONCLUSION

Our study showed that XCYS population was rich in genetic diversity and could be used as a better germplasm resource of *P. americana*. The study has clearly demonstrated the usefulness of SSR approach in determining the genetic variability among the *P. americana* germplasm. Identification of genetic variability among *P. americana* germplasm is critical to the conservation strategies as well as useful for designing appropriate breeding strategies for its genetic improvement.

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Statement of conflict of interest

The authors have declared no conflict of interests.

REFERENCES

Aichiyousfi, H., Bahri, B.A. and Medini, M., 2016. Genetic diversity and population structure of six species of *Capparis* in Tunisia using AFLP markers. *C. R. Biol*., **339**: 442-453. https://doi.org/10.1016/j.crvi.2016.09.001

Apostol, B.L., Reiter, P. and Miller, B.R., 1996. Population genetics with RAPD-PCR markers: the breeding structure of *Aedes aegypti* in Puerto Rico. *Heredity*, **76**: 325. https://doi.org/10.1038/hdy.1996.50

Balloux, F., Lugon-Moulin, N., 2010. The estimation of population differentiation with microsatellite markers. *Mol. Ecol*., **11**: 155-165. https://doi.org/10.1046/j.0962-1083.2001.01436.x

Barker, J.S.F., 1994. *A global protocol for determining genetic distance among domestic livestock breeds*. World Congress on Genetics Applied to Livestock.

Barrett, S.C. and Charlesworth, D., 1991. Effects of a change in the level of inbreeding on the genetic load. *Nature*, **352**: 522-524. https://doi.org/10.1038/352522a0

Booth, W., Bogdanowicz, S.M., Prodöhl, P.A., 2010. Identification and characterization of 10 polymorphic microsatellite loci in the German cockroach, *Blattella germanica*. *Mol. Ecol. Resour*., **7**: 648-650. https://doi.org/10.1111/j.1471-8286.2006.01663.x

Botstein, D., White, R.L. and Skolnick, M., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Human Genet*., **32**: 314-331.

Cariou, M.L., Solignac, M., Monnerot, M., 1990. Low allozyme and mtDNA variability in the island endemic species *Drosophila sechellia* (*D. melanogaster* complex). *Experientia*, **46**: 101-104. https://doi.org/10.1007/BF01955430

Chadee, D.D., 1990. *Aedes aegypti* surveillance in Tobago, West Indies (1983-88). *J. Am. Mosq. Contr. Assoc*., **6**: 148-150.

Clifford, M.N. and Willson, K.C., 1986. Coffee: botany, biochemistry and production of beans and beverage. *Econ. Bot*., **40**: 424-424. https://doi.org/10.1007/BF02859653

Cloarec, A., Rivault, C. and Cariou, M.L., 2010. Genetic population structure of the German cockroach, *Blatella germanica*: Absence of geographical variation. *Ent. Exp. Appl*., **92**: 311-319. https://doi.org/10.1046/j.1570-7458.1999.00552.x

Ellstrand, N.C. and Elam, D.R., 1993. Population genetic consequences of small population size: Implications for plant conservation. *Annu. Rev. Ecol. Syst*., **24**: 217-242. https://doi.org/10.1146/annurev.es.24.110193.001245

Estoup, A. and Angers, B., 1998. Microsatellites and minisatellites for molecular ecology: Theoretical and empirical considerations. *Adv. mol. Ecol. Nato Sci*., **38**: 69-75.

Jaramillo-Ramirez, G.I., Cárdenas-Henao, H. and González-Obando, R., 2010. Genetic variability of five *Periplaneta americana* L. (Dyctioptera: Blattidae) populations in southwestern Colombia using the AFLP molecular marker technique. *Neotrop. Ent*., **39**: 371. https://doi.org/10.1590/S1519-566X2010000300010

Lashermes, P., Trouslot, P. and Anthony, F., 1996. Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*. *Euphytica*, **87**: 59-64. https://doi.org/10.1007/BF00022965

Marshall, T.C., Slate, J. and Kruuk, L., 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol*., **7**: 639-655. https://doi.org/10.1046/j.1365-294x.1998.00374.x

Mishra, M.K., Nishani, S. and Gowda, M., 2014. Genetic diversity among Ethiopian coffee (*Coffea arabica* L.) collections available in Indian gene bank using sequence related amplified polymorphism markers. *Pl. Breed. Seed Sci*., **70**: 29-40. https://doi.org/10.1515/plass-2015-0011

Moazami-Guodarzi, K., Laloë, D. and Furet, J.P., 1997. Analysis of genetic relationships between 10 cattle breeds with 17 microsatellites. *Anim. Genet*., **28**: 338-345. https://doi.org/10.1111/j.1365-2052.1997.00176.x

Nei, M., 1972. Genetic distance between population. *Am. Nat*., **106**: 283-292. https://doi.org/10.1086/282771

Nei, M., 1973. Analysis of gene diversity in subdivided populations. *Proc. natl. Acad. Sci. U.S.A*., **70**: 3321-3323. https://doi.org/10.1073/pnas.70.12.3321

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583-590.

Nei, M., 1987. *Molecular evolutionary genetics*. Columbia University Press. https://doi.org/10.7312/nei-92038

Posso, C.E., González, R. and Cárdenas, H., 2003. Random amplified polymorphic DNA analysis of *Anopheles nuneztovari* (Diptera: Culicidae) from Western and Northeastern Colombia. *Mem. Inst. Oswaldo Cruz*, **98**: 469-476. https://doi.org/10.1590/S0074-02762003000400007

Powell, W., Machray, G.C. and Provan, J., 1996. Polymorphism revealed by simple sequence repeats. *Trends Pl. Sci*., **1**: 215-222. https://doi.org/10.1016/S1360-1385(96)86898-0

Raymond, M., 1995. GENEPOP version 3.4: Population genetics software for exact tests and ecumenicism. *J. Hered.*, **86**: 248-249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Reiter, P., Amador, M.A. and Anderson, R.A., 1995. Short report: Dispersal of *Aedes aegypti* in an urban area after blood feeding as demonstrated by rubidium-marked eggs. *Am. J. trop. Med. Hyg*., **52**: 177-179. https://doi.org/10.4269/ajtmh.1995.52.177

Rousset, F., 2008. Genepop'007: A complete re-implementation of the Genepop software for Windows and Linux. *Mol. Ecol. Resour*., **8**: 103-106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Runciman, D., Blacket, M.J. and Schmuki, C., 2010. Polymorphic population genetic markers for the Australian wood cockroach *Panesthia australis*. *Mol. Ecol. Resour*., **6**: 765-766. https://doi.org/10.1111/j.1471-8286.2006.01335.x

Schal, C. and Hamilton, R.L., 1990. Integrated suppression of synanthropic cockroaches. *Annu. Rev. Ent*., **35**: 521. https://doi.org/10.1146/annurev.en.35.010190.002513

Shuyskaya, E., Toderich, K. and Gismatullina, L., 2017. Genetic diversity of two annual Salsola species (Chenopodiaceae) among habitat types in desert plant communities. *Biologia*, **72**: 267-276. https://doi.org/10.1515/biolog-2017-0032

Slatkin, M., 2010. Gene flow in natural populations. *Climate Develop*., **2**: 9-13.

Tamaki, F.K., Pimentel, A.C. and Dias, A.B., 2014. Physiology of digestion and the molecular characterization of the major digestive enzymes from *Periplaneta americana*. *J. Insect Physiol*., **70**: 22-35. https://doi.org/10.1016/j.jinsphys.2014.08.007

Tautz, D., 1989. Hypervariabflity of simple sequences as a general source for polymorphic DNA markers. *Nucl. Acids Res*., **17**: 6463-6471. https://doi.org/10.1093/nar/17.16.6463

Weber, J.L. and May, P.E., 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet*., **44**: 388-396.

Wolff, S.L. and Jefferies, R.L., 1987. Morphological and isozyme variation in *Salicornia europaea* (s.l.) (Chenopodiaceae) in northeastern North America. *Canadian J. Bot*., **65**: 1410-1419. https://doi.org/10.1139/b87-195

Yeh, F.C., Yang, R.C. and Boyle, T., 1999. *POPGEN, version 1.32. Microsoft Windows-based freeware for population genetic analysis*. University of Alberta/CIFOR, Edmonton.