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Analysis of Genetic Diversity of the Higher and Lower Royal Jelly Producing Bee in China with Microsatellite Markers

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Abstract: Genetic diversity of Pinghu royal jelly bee (higher royal jelly producing bee) and Suwang No.1 bee (lower royal jelly producing bee) were evaluated with 23 microstaellite loci, the genetic variability within breeds and genetic differentiation between breeds were estimated, the results showed that in 23 microstaellite loci, 211 alleles were found, the number of alleles per locus ranged from 3-14, the average expected heterozygosity and PIC of all loci were 0.6894 and 0.6560, respectively. Mean numbers of alleles of Pinghu royal jelly bee and Suwang No.1 bee were 6.65 and 6.13, inbreeding index (FST), Reynolds' genetic distance and gene flow between two populations were 0.194, 0.2146 and 1.0447, respectively, these results indicated that the heterozygosity and the genetic diversity of 2 bee populations were very high.

Key words: Bee, royal jelly bee, microsatellite marker, genetic diversity

INTRODUCTION

China is not only the original area of honeybee, but also the country had a very long history of apiculture. In the end of the 19th centry, *Apis mellifera* was invited to China (Chen, 2001). Because the population has some excellenter characters than *Apis cerana cerana* who is aboriginal, *Apis mellifera* spread quickly across China and some new breeds were produced at the same time, such as Pinghu royal jelly bee and Suwang No.1 bee.

Pinghu royal jelly bee was cultivated in Pinghu of Zhejiang province, bred by hybridized *Apis mellifera* L. from Italy and *Apis mellifera* L. in Zhejiang. This breed has many fantastic peculiarities and the most outstanding one is higher royal production (Sun *et al*., 2003). Suwang No.1 bee was cultivated in Xuyi county of Jiangsu province in 1995, which was generalized all over China with its higher honey production, strong resistibility to illness and none bee larcenist. But this breed has much lower royal jelly production than Pinghu royal jelly bee although, they both are *Apis mellifera* L. (Ji and Zhang, 2003). Since, each one of these 2 breeds has its particular merits, they distribute broadly in Jiangsu and Zhejiang province. However, the genetic diversity research about these 2 breeds was infrequent.

With the characteristics of high polymorphism, locus specificity, abundance and random distribution over the genome and their codominant inheritance, microsatellites are currently most commonly used to assess population structure and diversity (Weigend and Romanov, 2001). According to FAO recommendations, determining classic genetic distances using neutral, highly polymorphic microsatellite markers is the method of choice for investigating genetic relationships and breed differentiation. This methodology also provides information for establishing preservation priorities for livestock breeds (Barker, 1999).

The aim of this study was to analyze genetic diversity in these two *Apis mellifera ligustica* populations with 23 microsatellite markers and to evaluate their genetic structure. The results may be useful to understand genetic differentiation of this important genetic resource and contribute to a more efficient conversation.

MATERIALS AND METHODS

Experimental populations: Ninty two individuals from Pinghu royal jelly bee and 99 individuals from Suwang No.1 bee were genotyped. DNA was extracted according to the reports by Ji *et al*. (2005) and then preserved in -20°C after tested content and purity.

Genotyping: Twenty three microsatellite markers (Table 1) spread across the honeybee genome were used for genotypes. The primers are selected according to NCBI (http://www.ncbi.nlm.nih.gov) and report by Michel *et al*. (2003). PCR products were obtained in a 20 μL volume

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Table 1: The location of 23 microsatellite loci in chromosome or linkage group and condition of PCR

Locus	GenBank accession No.	Chromosome or linkage group	Concentration of Mg^+	Annealing temperature (°C)
AP243	AJ509466	Chr LG1	2.2	57.5
Ag005a	AJ509722	Chr LG1	2.0	55.0
Ap049	AJ509334	Chr LG1	2.2	55.6
AP226	AJ509455	Chr LG1	2.0	55.6
AC306	AJ509721	Chr LG2	2.0	55.6
AP274	AJ509486	Chr LG3	2.0	55.0
Ap043	AJ509329/AJ509667	Chr LG3	2.2	56.5
AP313	AJ509504	Chr LG4	2.0	57.0
A113	AJ509290	Chr LG6	2.0	58.2
A107	AJ509287	Chr LG7	2.0	57.0
A024d	AJ509241	Chr LG7	2.0	57.0
A014	AJ509239	Chr LG8	2.0	55.6
A088	AJ509283	Chr LG8	1.6	58.0
AC011	AJ509637	Chr LG9	1.8	57.0
BI366	BI516839	Chr LG11	1.6	57.0
AT101	AJ509549	Chr LG12	2.0	55.6
Ap297	AJ509499	Chr LG12	2.2	56.5
Ap085	AJ509359	Chr LG12	2.0	56.5
At003	AJ509505	Chr LG13	2.0	55.6
A035	AJ509251	Chr LG14	2.0	53.4
A028	AJ509244	Chr LG14	2.0	55.0
Ap068	AJ509351	Chr LG15	2.0	55.6
AG005C	AJ509723	Chr LG16	2.0	55.0

using thermal cycler. Each PCR tube contained 50 ng of genomic DNA, 2.0 μL of 10×buffer, 1.2~2.0 μL of 25 mmol L^{-1} $MgCl_2$, 0.5 μL of 10 mmol μL^{-1} dNTP, 1 μL of both 10 pmol μL^{-1} forward primer and reverse primer, 5 U μL^{-1} Taq DNA Polymerase 0.2 μL. The amplification involved initial denaturation at 95°C (5 min), 35 cycles of denaturation at 95°C (50 sec), annealing temperature varying between 50 and 60°C (50 sec) and extension at 72°C (50 sec), followed by final extension at 72°C (10 min). DNA fragments were scored on 8% polyacrylamide gel using a LI-COR automated DNA analyzer (LI-COR Biotechnology Division, Lincoln, NE68504). Electrophoregram processing and allele-size scoring was performed with the RFLP scan package (Scanalytics, Division of CSP, Billerica, MA).The primers are selected according to NCBI (http://www.ncbi. nlm.nih.gov) and report by Michel *et al.* (2003).

Statistical analysis

Genetic diversity: Allele frequencies, the observed and expected heterozygosity (Ho and He) (Nei, 1987) for each population across the loci and that for each locus across populations were estimated with Microsatellite-Toolkit for Excel. Polymorphism Information Content (PIC) for each locus and each breed was obtained according Botstein *et al.* (1980):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2$$

where:

n = The number of alleles
p_i = Frequency of the allele i
p_j = Frequency of the allele j

Genetic differentiation: The F-statistics indices (Wright, 1978), were estimated in the form of F, θ and f, the sample-based, respective estimators of these parameters proposed by Weir and Cockerham (1984), as implemented in FSTAT program. Significance of the F-statistics was determined from permutation tests with the sequential Bonferroni procedure applied over loci (Hochberg, 1988). As a measure of deviation from Hardy-Weinberg equilibrium, the F_{IS} value was calculated and type-I error probability was computed. The F_{ST} values among pairs of breeds were calculated with GENEPOP program (Raymond and Rousset, 1995). Gene flow between populations, defined as the number of reproductively successful migrants per generation (Nm), was estimated by the methods based on the n island model of population structure. The estimate was based on the relationship:

$$F_{ST} = 1/(4\,Nm + 1)$$

where:

N = The effective population size
m = The migration rate
F_{ST} = The mean F_{ST} value calculated over all loci (Slatkin and Barton, 1989)

The Reynolds *et al.* (1983) genetic distance between populations was calculated, based on F_{ST} values.

RESULTS

A total of 211 alleles were detected in two *Apis mellifera ligustica* populations in China with 23 microsatellite markers. Total number of alleles, size of

Table 2: Names, total number of alleles, size of allele, expected heterozygosity and PIC of 23 microsatellite markers

Locus	Chromosome	No. alleles	Size of alleles(bp)	Expected heterozygosity
AP243	5	253-366	0.2516	0.2317
Ag005a	5	72-90	0.5081	0.4523
Ap226	9	240-260	0.8004	0.7705
Ap049	10	90-166	0.7648	0.7269
AC306	8	150-180	0.7961	0.7673
AP274	10	102-209	0.4420	0.4091
Ap043	11	106-160	0.6277	0.5962
AP313	6	366-400	0.5970	0.5172
A113	13	186-226	0.8605	0.8431
A107	14	127-188	0.8733	0.8578
A024d	7	68-98	0.6108	0.5766
A014	9	184-247	0.5645	0.5394
A088	14	116-147	0.8826	0.8689
AC011	12	98-136	0.8487	0.8287
BI366	3	116-120	0.5342	0.4374
AT101	7	253-281	0.6558	0.6029
Ap297	4	115-150	0.3603	0.3333
Ap085	14	180-198	0.8646	0.8474
At003	12	190-217	0.8811	0.8668
A035	10	82-201	0.8155	0.7874
A028	9	114-116	0.7348	0.7010
Ap068	9	148-213	0.7463	0.7105
Ag005C	10	102-148	0.8347	0.8146
Mean	9.1739		0.6894	0.6560
SD	(3.2144)		(0.1807)	(0.1895)

SD: Standard Deviation for mean number of alleles; He and PIC, were given in parentheses

Table 3: Mean number of alleles per locus, mean heterozygosity (He and Ho) for 2 populations

Population	No. alleles	Average He	Average Ho
Pinghu royal jelly bee	6.65±2.77	0.5940±0.2191	0.2513±0.2258
Suwang No. 1 bee	6.13±2.28	0.6342±0.1644	0.2237±0.2218

alleles, Expected heterozygosity (He) and mean Polymorphic Information Content (PIC) for each locus across 2 populations were listed in Table 2.

The number of alleles per locus ranged from 3 (BI366) to 14 (A107, A088, A085) and the average number of the alleles observed in 23 microsatellite loci was 9.1739+3.2144. Across populations, locus AP243 had the lowest He, 0.2516 and the lowest PIC, 0.2317, however, the locus A088 had the highest He and PIC value, 0.8826 and 0.8689, respectively.

The average number of alleles per locus, expected and observed heterozygosity and for each population across 23 loci were shown in Table 3.

Average number of alleles/locus was 6.65 in Pinghu royal jelly bee and 6.13 in Suwang No.1 bee. Both 2 populations showed relative large heterozygosity. Across 23 loci, the lower value 0.5940 of heterozygosity was obtained for the Pinghu royal jelly bee and the higher 0.6342 was found for Suwang No.1 bee.

The results from F-statistics analysis was shown in Table 4. The negative FIS values of some populations indicated an excess of heterozygous genotypes with respect to the expected value. For the 2 populations, inbreeding index (FST) was 19.4% ($p<0.01$), there were 20 loci supported this result. Reynolds *et al.* (1983) genetic distance and gene flow between 2 populations were 0.2146 and 1.0447, respectively.

DISCUSSION

The 23 microsatellite markers used in the present study are randomly distributed across 14 chromosomes or linkage groups in the honeybee genome, so the data had certain commparability and representativeness. The Polymorphism Information Content (PIC) value is a good measure of the polymorphisms of gene fragment, while PIC >0.5, the locus is a highly polymorphic locus; while 0.25< PIC <0.5, the locus is a medium polymorphic locus; while PIC <0.25, the locus is a low polymorphic locus (Vanhala *et al*., 1998). Meanwhile, PIC value is related to the availability and utilization efficiency of a marker, the higher PIC value of the marker, the higher heterozygote frequency in one population, as well as the more genetic information it provides. In this study, 18 loci among 23 microsatellite loci exhibited high polymorphic, while 4 loci showed medium polymorphic, mean PIC value across all loci exceeded 0.5, which could provide enough information for the assessment of genetic diversity.

Effective number of alleles is also a good measure of the genetic variation, especially in conservation genetics study. Sometimes its effect on populations is put more emphasis, but effective number of alleles is easy to be affected by sample size (Maudet *et al*., 2002). The average number of the alleles was 9.1739 across 23 microsatellite loci in the present study, which indicated that the sample size was enough. On the other hand, this result also indicated that the polymorphism information content provided by these 23 microsatellite loci in 2 populations was rich and the distribution of the allelic frequency was rather even. Therefore, using effective number of alleles to analysis genetic diversity is more effective and reliable.

Gene heterozygosity, also called gene diversity, is a suitable parameter for investigating genetic variation. Ott (2001) gave a definition that a polymorphic locus must have at least 0.10 heterozygosity. All 23 microsatellite loci in this study had high polymorphism with a mean expected heterozygosity, 0.6894, showing a high degree of genetic diversity and relative high selection potential. Mean expected heterozygosity can approximately reflect the variation of genetic structure, Suwang No.1 bee had the higher genetic variability (0.6342) than Pinghu royal jelly bee (0.5940), but the difference was not distinct.

In our study, on average, the genetic differentiation (F_{ST}) among breeds was 19.4% (Table 4), a relative high value and extremely significant ($p<0.001$), which indicated that there is a great differentiation among two

Table 4: The results from F-statistics analysis

Locus	$F_{IT} = F$	$F_{ST} = \theta$	$F_{IS} = f$
AP243	0.148*	0.038	0.114
Ag005a	0.190***	0.006**	0.186***
Ap226	0.852***	0.104***	0.834***
Ap049	0.920***	0.076***	0.913***
AC306	0.560***	0.238***	0.423***
AP274	0.079	0.002***	0.078
Ap043	0.755***	0.138**	0.715***
AP313	0.704***	0.149***	0.652***
A113	0.656***	0.193***	0.574***
A107	0.626***	0.095***	0.587***
A024d	0.623***	0.132***	0.565***
A014	0.799***	0.105***	0.775***
A088	0.707***	0.101***	0.674***
AC011	0.849***	0.268***	0.793***
BI366	0.993***	0.638***	0.981***
AT101	0.743***	0.267***	0.650***
Ap297	-0.098	0.094***	-0.212
Ap085	0.678***	0.170***	0.612***
At003	0.959***	0.222***	0.947***
A035	-0.170	0.073***	-0.262
A028	0.994***	0.523***	0.988***
Ap068	0.826***	0.124***	0.801***
Ag005C	0.793***	0.267***	0.717***
Mean	0.675***	0.194***	0.596***
SD	(0.062)	(0.033)	(0.070)

F: Total inbreeding estimate; F_{ST}, measure of population differentiation; f, within-population inbreeding estimate; Mean estimates from jack-knife over loci, standard deviations are given in parentheses; Significance of F-statistics was done using Bonferroni permutations based on 1000 resamplings; *p<0.05; **p<0.01; ***p<0.001

Apis mellifera L. populations. It is clear that about 19.4% of the total genetic variation corresponds to differences of populations and the remaining 80.6% is the result of differences among individuals. All loci contribute to this differentiation significantly.

The coefficient F_{IS}, which indicates the degree of departure from random mating, positive F_{IS} values mean a significant deficit of heterozygotes, while the negative F_{IS} values indicate an excess of heterozygous genotypes with respect to the expected value. In this study, average of F_{IS} was 0.596. In addition, all loci showed significant deficit of heterozygotes except AP297 and A035. Two reasons maybe contribute to the deficit of heterozygotes for these 23 loci: first, the locus may be under selection (genetic hitchhiking effect) with some morphological or productive traits of selective interest; secondly, 'null alleles' may be present.

CONCLUSION

In conclusion, the heterozygosity and the genetic diversity of two bee populations were very high. Suwang No.1 bee had the higher genetic variability than Pinghu royal jelly bee, but the difference was not distinct. There is a great differentiation among 2 *Apis mellifera* L. populations.

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