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Full Length Research Paper

Genetic diversity and population structure of Chinese honeybees (*Apis cerana*) under microsatellite markers

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Using 21 microsatellite markers and PCR method, the polymorphisms of 20 *Apis cerana* honeybee populations across China was investigated and the genetic structure and diversity of the populations were explored. The results showed that 507 alleles (mean 24.14 per locus, ranging from 13 to 45) were observed in 842 honeybees. Wuding bee had the highest level of heterozygosity (0.695), and the lowest estimate was 0.207 for Changbai bee. The global heterozygote deficit across all populations (*Fit*) amounted to 0.776. About 42.3% of the total genetic variability originated from differences between breeds, with all loci contributing significantly to the differentiation. An unrooted consensus tree using the Neighbour-Joining method and pair-wise distances showed that 6 populations from Eastern China clustered together. The structure analysis indicated that the 6 populations were separated first. These findings demonstrated that the 6 honeybee populations had close genetic relationships.

Key words: *Apis cerana*, microsatellite, polymorphism, genetic structure.

INTRODUCTION

Chinese indigenous honeybee (*Apis cerana*) resources have a long apiculture history and survive under diversified geographical conditions. Recently their population size rapidly decreased for the reason of environmental pollution and competition from *Apis mellifera* introduced into China since 1896 (Yang, 2005).

With the characteristics of locus specificity, rich polymorphism, abundant and random distribution over the genome, and their co-dominant inheritance, microsatellites are currently most commonly used to assess population structure and diversity (Chapman et al., 2008; Delaney et al., 2009; Soland-Reckeweg et al., 2009; Bourgeois and Rinderer, 2009; Kence et al., 2009). 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 work was to evaluate the genetic diversity and estimate the genetic structure of Chinese indigenous honeybee populations using 21 microsatellite markers. The results may help to understand the genetic differentiation of *A. cerana* in China and contribute to more efficient conversation strategies.

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MATERIALS AND METHODS

Experimental populations

842 honeybees from 20 populations (*A. cerana*) were sampled and analyzed in this study. The information about the populations is presented in Table 1.

Microsatellite DNA genotyping

The microsatellite DNA markers for 21 microsatellite loci (Table 2) were used to explore the genotypes of each population. The location of 21 microsatellite loci in the chromosome and condition of PCR were illustrated below.

Primers were selected according to Genbank and report by Solignac et al. (2003). PCR products were obtained in a 20 µl reaction mixture 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 $MgCl_2$, 5 µl of 10 mmol/µl dNTP, 1 µl of both 10 pmo1/µl forward primer, 10 pmo1/µl reverse primer and 0.2 µl of 5 U/µl Taq DNA polymerase. PCR was predenatured at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 50 s, annealing at the optimal temperature

Table 1. Collecting data of 20 populations of *A. cerana* in China.

Population	Code	Collection date	Collection locality	Position	Number of sample
Diqing population	DQ	2007 - 09 - 10	Diqing, Yunnan	27° 40′ N, 99° 55′ E	33
Wuding population	WD	2007 - 06 - 21	Wuding, Yunnan	25° 50′ N, 102° 15′ E	50
Xishuangbanna population	XS	2007 - 09 - 13	Xishuangbann, Yunnan	21° 6′ N, 100° 07′ E	30
A-ba population	AB	2008 - 08 - 12	Maerkang,Sichuan	31° 28′ N, 101° 50′ E	41
Beijing population	BJ	2007 - 07 - 12	Fangshan,Beijing	39° 56′ N, 116° 20′ E	33
Tiansui population	TS	2007 - 08 - 09	Maijishan,Gansu	34° 4′ N, 106° 0′ E	59
Conghua population	CH	2007 - 08 - 12	Conghua, Guangdong	23° 57′ N, 113° 55′ E	30
Nanling population	NL	2007 - 08 - 10	Nanning, Guangxi	22° 50′ N, 118° 11′ E	32
Hainan population	HN	2007 - 09 - 01	Haikou, Hainan	20° 03′ N, 110° 35′ E	53
Fengxian population	LF	2007 - 05 - 10	Linfeng, Hunan	29° 28′ N, 111° 36′ E	31
Changbaishan population	CB	2007 - 08 - 10	Dunhua, Jilin	41° 55′ N, 127° 55′ E	30
Xinchen population	XC	2008 - 07 - 11	Xingcheng , Liaoning	40°34′ N, 120° 44′ E	30
Qingling population	QL	2008 - 04 - 09	Baoji, Shanxi	34° 35′ N, 107° 42′ E	30
Tibet population	XZ	2008 - 6 - 23	Bomi Tibet	24° 59′ N, 121° 67′ E	30
Huangshan population	HS	2007 - 07 - 18	Huangshan, Anhui	29° 57′ N, 118° 14′ E	76
Wuyishan population	WX	2007 - 04 - 20	Wuyishan, Fujian	27° 45′ N, 118° 02′ E	50
Nanchan population	NC	2007 - 05 - 22	Nanchan, Jiangxi	31° 22′ N, 119° 49′ E	58
Feixian population	FX	2007 - 08 - 20	Feixian, handong	35° 15′ N, 117° 58′ E	30
Yixing population	YX	2006 - 09 - 28	Yixing, Jiangsu	31° 22′ N, 119° 49′ E	81
Tonglu population	TL	2007 - 07 - 19	Tonglu, Zhejiang	29° 50′ N, 119° 34′ E	35

(Table 2) for 50 s, extension at 72°C for 50 s, and with a final extension at 72°C for 10 min. PCR products 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).

Statistical analysis

The total number of alleles, allele frequencies, average number of alleles per locus, observed (*Ho*) and expected heterozygosity (*He*) for each population across the loci, were estimated with microsatellite-toolkit for Excel (Park, 2001).

Population differentiation was estimated by Wright's (1978) fixation indice F_{IT}, F_{ST} and F_{IS} in the form of F, θ and f, respectively, for each locus across populations according to the variance based on the method by Weir and Cockerham (1984) using FSTAT software (Version 2.9.3, Goudet, 2002). The significance of the F-statistics was determined by permutation tests with the sequential Bonferroni procedure (Hochberg, 1988). The extent of inbreeding was further studied with GENEPOP software (Raymond and Rousset, 1995) by estimation of the *Fis* values and their significance level within each of the populations.

Pair-wise *Fst* values were computed for all the combinations of the 15 populations using GENEPOP software. Gene flow between populations, defined as the number of reproductively successful migrants per generation (*Nm*), was estimated based on the n island model of population structure (Slatkin and Barton, 1989). The estimate was based on the relationship $Fst = 1/(4Nm+1)$, where *N* is the effective population size, *m* is the migration rate, and *Fst* is calculated as mean over loci.

The software structure (Pritchard et al., 2000) was utilized through a Monte Carlo Markov chain (MCMC) algorithm to assess the presence of a structure underlying the genetic information provided by the genetic markers. The software was run 100 times with 50,000 iterations after a burn-in period of 20,000 iterations, and for each number of genetic clusters (K) a priori was chosen. K values for genetic structure ranged from two to seven. A pair-wise comparison of the hundred solutions for each K value was done using SIMCOEFF software (Rosenberg et al., 2002). Values with over 95% similarity were considered as identical. The most frequent solution for each K was taken as the most probable clustering and visualized using district software (Rosenberg, 2004). Additional sub-clustering were carried out in those subsets of the populations which did show population differentiation at level K = 6. An unrooted Neighbor- Joining cladogram (Saitou and Nei, 1987) based on Reynolds' distance matrix between populations was then constructed with the evaluation by 1000 bootstraps across the set of loci.

RESULTS

Genetic diversity of 20 *Apis cerana* populations

A total of 507 alleles were detected in the 20 *A. cerana* populations. All these microsatellite loci were polymorphic (Table 3). The number of alleles per locus ranged from 13 (BI366) to 45 (AC011), and the average number of the alleles observed was 24.143.

The fixation coefficients of subpopulations of these 21 loci within the total population, measured as F_{ST} value, varied from 0.203 (AC011) to 0.762 (AG005C), with a mean of 0.423 (Table 4). All loci contributed significantly to this differentiation. The global deficit of heterozygotes across populations (F_{IT}) amounted to 0.776. Mean F_{IS} was 0.612 ($P < 0.001$) within populations. All the loci showed

Table 2. The microsatellite DNA markers for the 21 microsatellite loci.

Microsatellite locus	GenBank accession	Chromosome	Mg^{2+} (mmol/l)	Annealing temperature (°C)
AP243	AJ509466	Chr LG1	2.2	57.5
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
Ap043	AJ509329 / AJ509667	Chr LG3	2.2	56.5
AP313	AJ509504	Chr LG4	2.0	57
A113	AJ509290	Chr LG6	2.0	58.2
A107	AJ509287	Chr LG7	2.0	57
A024d	AJ509241	Chr LG7	2.0	57
A014	AJ509239	Chr LG8	2.0	55.6
A088	AJ509283	Chr LG8	1.6	58
AC011	AJ509637	Chr LG9	1.8	57
BI366	BI516839	Chr LG11	1.6	57
AT101	AJ509549	Chr LG12	2.0	55.6
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
Ap068	AJ509351	Chr LG15	2.0	55.6
AG005C	AJ509723	Chr LG16	2.0	55

Table 3. Expected heterozygosity (He) and PIC based on the 21 microsatellite markers.

Locus	Number of allele	Size of allele (bp)	Expected heterozygosity	PIC
AP243	21	242 - 366	0.8461	0.8309
AP049	21	106 - 184	0.8379	0.8269
AP226	21	222 - 349	0.9044	0.8966
AC306	19	104 - 184	0.8049	0.7794
AP274	30	78 - 230	0.8704	0.8580
AP043	39	106 - 217	0.9142	0.9103
AP313	15	281 - 449	0.7501	0.7191
A113	27	166 - 240	0.9105	0.9045
A107	23	124 - 250	0.8775	0.8661
A024d	18	64 - 144	0.8818	0.8716
A014	17	85 - 250	0.8045	0.7818
A088	21	116 - 166	0.8142	0.7923
AC011	45	91 - 315	0.9626	0.9616
BI366	13	106 - 172	0.8332	0.8119
AT101	21	234 - 400	0.8700	0.8596
AP085	37	120 - 238	0.9356	0.9322
AT003	32	178 - 250	0.9559	0.9546
A035	26	82 - 267	0.8648	0.8545
A028	23	84 - 160	0.8711	0.8583
AP068	23	119 - 213	0.8903	0.8815
AG005C	15	98 - 160	0.8471	0.8319
Mean	24.1430 (8.3320)		0.8689 (0.0525)	0.8564 (0.0603)

Standard deviations for mean number of alleles, He and PIC, were given in parentheses.

Table 4. Hardy-Weinberg equilibrium analysis and *F*-statistics of the loci.

Locus	F_{IT} = F	F_{ST} = θ	F_{IS} = f	Number of population deviation from H-W equilibrium
AP243	0.724***	0.306***	0.601***	16
AP049	0.705***	0.373***	0.529***	13
AP226	0.834***	0.504***	0.666***	12
AC306	0.932***	0.552***	0.848***	13
AP274	0.762***	0.661***	0.296***	6
AP043	0.574***	0.226***	0.450***	18
AP313	0.857***	0.454***	0.738***	18
A113	0.719***	0.311***	0.592***	15
A107	0.951***	0.274***	0.933***	19
A024d	0.457***	0.504***	- 0.095	13
A014	0.879***	0.717***	0.572***	8
A088	0.975***	0.608***	0.936***	11
AC011	0.681***	0.203***	0.600***	20
BI366	1.000***	0.708***	1.000***	11
AT101	0.966***	0.465***	0.937***	15
AP085	0.650***	0.232***	0.544***	18
AT003	0.724***	0.215***	0.648***	20
A035	0.450***	0.380***	0.114***	11
A028	0.816***	0.270***	0.748***	19
AP068	0.751***	0.294***	0.647***	19
AG005C	0.965***	0.762***	0.853***	9
Mean	0.776*** (0.035)	0.423*** (0.04)	0.612*** (0.053)	14

Mean estimates from jack-knife over loci, standard deviations are given in parentheses; *P < 0.05, **P < 0.01; ***P < 0.001.

significant deficit of heterozygotes (P < 0.001) except one (A024d) with excess of heterozygotes.

Average number of alleles per locus ranged from 1.81 in Changbai population to 6.30 in Nangchang population (Table 5). The lowest estimate of expected heterozygosity (0.2066) was obtained for Changbai population, while the highest one (0.6957) was found in Wuding population.

Genetic distances and clustering of breeds

The *Nm* value ranged from 0.128 between NC and BJ to 12.376 between DQ and WD (Table 6). Reynolds' distance values varied between 0.020 (between DQ and WD) and 1.085 (between NC and BJ).

The results of the clustering analysis using structure were displayed in Figure 1. At K = 2, two main groups were formed. At this K value, populations from Eastern China formed one group and the other populations were grouped into a different cluster.

At K = 3, the Eastern China cluster maintained its structure as formed as K = 2, while the others were divided into two group (Qinling bee, Tibetan bee, Xishuangbanna bee, Tianshui bee, Hainan bee and Wuding bee formed one group). At K = 4, the Eastern China populations clustered into two distinct clusters, separating the Huangshan bee, Tonglu bee and Yixing bee from the rest. At K = 5, Fengxian bee, Nanning bee and Conghua bee made up their own separate cluster. The Tianshui bee, Hainan bee and Wuding bee split off to form its own cluster at K = 6. All populations showed complicated genetic bases at K = 7.

Since the clustering algorithm implemented in the structure is computer intensive, the work did not proceed with higher K values in the total set of populations. Instead, subsets of populations which did not show population separation at level K = 6 were analyzed. In the first subset encompassing populations Conghua, Fengxian and Nanning, the three populations did not separate from each other at all from K = 2 to K = 3. In the second subset including Beijing, Changbai and Diqing, populations formed a distinct cluster first (K = 2) followed by Changbai bee (K = 3). In the third and fourth subsets Tianshui, Hainan, Wuding, Qinling, Tibetan and Xishuangbanna populations always appeared as a mixture population. In the fifth subsets, Wuyi separated first (K = 2). In the sixth subsets, Yixing separated first (K = 2) and Huangshan appeared as a mixture population.

The Neighbour-Joining (NJ) tree derived from the Reynolds' genetic distance showed that Tianshui, Hainan,

Table 5. Mean heterozygosity (He and Ho) for the 20 *Apis cerena* population.

Population	Number of allele (mean ± standard deviation)	Average He (mean ± standard deviation)	Average Ho (mean ± standard deviation)
DQ	2.52 ± 1.25	0.3665 ± 0.0552	0.1880 ± 0.0156
WD	5.67 ± 2.48	0.6957 ± 0.0275	0.2517 ± 0.0175
XS	4.62 ± 1.96	0.5963 ± 0.0499	0.1748 ± 0.0156
AB	2.38 ± 1.43	0.3260 ± 0.0691	0.0778 ± 0.0107
BJ	1.90 ± 0.94	0.2484 ± 0.0553	0.1428 ± 0.0134
TS	5.76 ± 3.19	0.5971 ± 0.0554	0.3330 ± 0.0188
CH	3.14 ± 2.29	0.3537 ± 0.0307	0.0623 ± 0.0097
NL	2.38 ± 1.60	0.3162 ± 0.0722	0.1132 ± 0.0121
HN	6.14 ± 3.05	0.6641 ± 0.0326	0.2758 ± 0.0137
LF	2.86 ± 2.41	0.3260 ± 0.0740	0.1007 ± 0.0119
CB	1.81 ± 0.93	0.2066 ± 0.0509	0.1512 ± 0.0143
XC	2.43 ± 1.43	0.3784 ± 0.0678	0.0903 ± 0.0115
QL	3.90 ± 1.70	0.5342 ± 0.0548	0.1471 ± 0.0142
XZ	3.95 ± 1.66	0.5625 ± 0.0566	0.0978 ± 0.0121
HS	6.26 ± 3.08	0.6099 ± 0.0443	0.2865 ± 0.0111
WX	4.09 ± 3.50	0.4280 ± 0.0630	0.1991 ± 0.0121
NC	6.30 ± 3.34	0.6329 ± 0.0415	0.1873 ± 0.0110
FX	4.57 ± 2.35	0.5732 ± 0.0543	0.2526 ± 0.0173
YX	4.65 ± 3.13	0.4900 ± 0.0555	0.2871 ± 0.0107
TL	5.39 ± 2.21	0.6203 ± 0.0385	0.2308 ± 0.0157

Ho, average number of alleles per locus, observed; He, expected heterozygosity for each population across the loci.

Wuding and the 6 populations from Eastern China formed one cluster; Beijing, Changbai and Xingcheng populations from Northern China formed cluster and Xishuangbanna, Tibetan, Qinling and A-ba populations from Western China, fell together with Fengxian, Nanning populations from Southern China; Conghua population was outside of the tree (Figure 2).

The application of Rousset's isolation by distance method, as implemented in GENEPOP program, yielded the parameters α and β in the regression and *Fst*/ (1-*Fst*) = 0.209 + 0.084 ln(d) (Figure 3). However, regression failed to provide enough support for a significant correlation between the genetic and geographical pair wise distances, as indicated by Mantel's test (P = 0.055).

DISCUSSION

Genetic variability within populations

The 21 microsatellite markers used in the present study were selected from NCBI (National Center for Biotechnology Information). The polymorphism information content (PIC) value is a good measure of the polymorphisms of gene fragment, when PIC > 0.5, the locus is a highly polymorphic locus; while when 0.25 < PIC < 0.5, the locus is a medium polymorphic locus and when 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 the PIC value of the marker, the higher the heterozygote frequency in one population, as well as the more genetic information it provides. In this study, all of the 21 microsatellite loci exhibited high 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 put more emphasis, but effective number of alleles is easily affected by sample size (Maudet et al., 2002). The average number of the alleles was 24.1430 for the 21 microsatellite loci in this study, which indicated that the sample size was enough. On the other hand, the polymorphism information content was rich at the 21 microsatellite loci in the 20 populations; and the distribution of the allelic frequency in all loci was rather even. Therefore, using effective number of alleles to analyze 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 a heterozygosity of at least 0.10. All the 21 microsatellite loci in this study had high polymorphisms with a mean expected heterozygosity of 0.8378, showing a high degree of genetic diversity and relative high selection

Table 6. Reynolds' genetic distances, D_R (upper triangle) and the gene flow, *Nm* (lower triangle) among populations.

Population	DQ	WD	XS	AB	BJ	TS	CH	NL	HN	LF	CB	XC	QL	XZ	HS	WYS	NC	FX	YX	TL
DQ		0.020	0.026	0.862	0.912	0.593	0.467	0.539	0.502	0.868	0.778	0.596	0.604	0.622	0.622	0.720	0.954	0.661	0.441	0.551
WD	12.376		0.058	0.868	0.891	0.610	0.476	0.556	0.540	0.889	0.789	0.609	0.613	0.634	0.632	0.724	0.961	0.654	0.465	0.586
XS	9.365	4.159		0.807	0.874	0.549	0.432	0.490	0.465	0.830	0.730	0.565	0.579	0.584	0.574	0.693	0.914	0.650	0.416	0.516
AB	0.183	0.181	0.201		0.567	0.682	0.600	0.699	0.709	0.899	0.895	0.678	0.681	0.736	0.814	0.796	1.051	0.714	0.539	0.714
BJ	0.168	0.174	0.179	0.328		0.734	0.628	0.774	0.796	1.057	0.986	0.712	0.658	0.780	0.812	0.804	1.085	0.918	0.579	0.790
TS	0.309	0.297	0.342	0.256	0.231		0.145	0.462	0.445	0.645	0.630	0.356	0.355	0.351	0.471	0.413	0.587	0.597	0.183	0.425
CH	0.420	0.410	0.463	0.304	0.286	1.606		0.360	0.347	0.538	0.540	0.320	0.318	0.274	0.395	0.371	0.485	0.480	0.180	0.325
NL	0.350	0.336	0.395	0.247	0.214	0.425	0.577		0.067	0.482	0.631	0.446	0.479	0.402	0.470	0.591	0.732	0.491	0.278	0.087
HN	0.383	0.349	0.422	0.242	0.206	0.446	0.603	3.590		0.506	0.623	0.421	0.451	0.381	0.443	0.571	0.711	0.461	0.265	0.037
LF	0.181	0.175	0.193	0.172	0.133	0.276	0.351	0.404	0.380		0.760	0.592	0.609	0.612	0.719	0.725	0.890	0.665	0.443	0.486
CB	0.212	0.208	0.233	0.173	0.149	0.285	0.349	0.284	0.289	0.220		0.595	0.640	0.654	0.761	0.755	0.939	0.782	0.479	0.603
XC	0.307	0.298	0.329	0.258	0.241	0.586	0.664	0.445	0.478	0.310	0.307		0.397	0.305	0.414	0.438	0.493	0.569	0.297	0.412
QL	0.302	0.295	0.319	0.256	0.269	0.587	0.667	0.407	0.439	0.298	0.279	0.514		0.275	0.402	0.212	0.512	0.573	0.352	0.420
XZ	0.290	0.283	0.315	0.230	0.212	0.594	0.793	0.505	0.540	0.296	0.271	0.700	0.791		0.416	0.384	0.559	0.589	0.294	0.335
HS	0.290	0.283	0.322	0.199	0.199	0.416	0.516	0.417	0.449	0.238	0.219	0.488	0.505	0.485		0.520	0.704	0.698	0.356	0.445
WY	0.237	0.235	0.250	0.206	0.202	0.488	0.556	0.310	0.325	0.235	0.222	0.455	1.060	0.533	0.366		0.572	0.681	0.434	0.550
NC	0.157	0.155	0.167	0.134	0.128	0.313	0.400	0.232	0.241	0.174	0.161	0.393	0.374	0.334	0.245	0.324		0.899	0.555	0.676
FX	0.267	0.271	0.273	0.240	0.166	0.306	0.406	0.395	0.427	0.265	0.211	0.326	0.323	0.312	0.247	0.256	0.172		0.431	0.458
YX	0.451	0.423	0.485	0.350	0.318	1.242	1.265	0.780	0.825	0.449	0.407	0.724	0.593	0.732	0.585	0.460	0.337	0.464		0.266
TL	0.340	0.314	0.370	0.240	0.208	0.472	0.652	2.758	6.714	0.399	0.302	0.490	0.478	0.628	0.446	0.341	0.259	0.430	0.820	

potential. Mean expected heterozygosity can approximately reflect the variation of genetic structure. Wuding population had the highest genetic variability, and the Changbai population had the lowest one. This might be due to the fact that there are rich natural resources with a favorable condition for genetic differentiation in Wuding of Yunnan province. The genetic basis of Wuding population is complicated. Some gene flow between Wuding population and other populations found in neighbouring regions possibly exist. This explains the highest Nm values (12.376) of Wuding population and Diqing population. The special geographical conditions limit the Changbai population to a relatively isolated region. The region is surrounded by mountains and these may act as barriers to gene flow. The population therefore had less opportunity for genetic exchange with other populations as indicated by the lower Nm values (from 0.149 to 0.409). Darvill et al. (2006) reported that isolated populations showed lower genetic diversity. Low genetic diversity may cause homozygous sex alleles in bees which can produce diploid drones, affecting the viability of bees. In the apicultural history of China, most honey used as ancient tribute to emperor was produced by Changbai bee. This study prompted the use of more effective measures to protect this valuable resource. Beijing bee is a relative isolation population like Changbai bee (Yang, 2001). In recent years, its distribution area as well as the population size decreased due to the destruction of plants in northern China. The low genetic diversity detected in this study reflected this situation, which indicated that priorities for the conservation of the populations distributed in Northern China should be conducted.

In the exact test for deviation from Hardy-Weinberg equilibrium, more or less populations showed significant deviation for all loci. Departures from HWE maybe due to a variety of causes: small population size, assortative mating system (inclu-

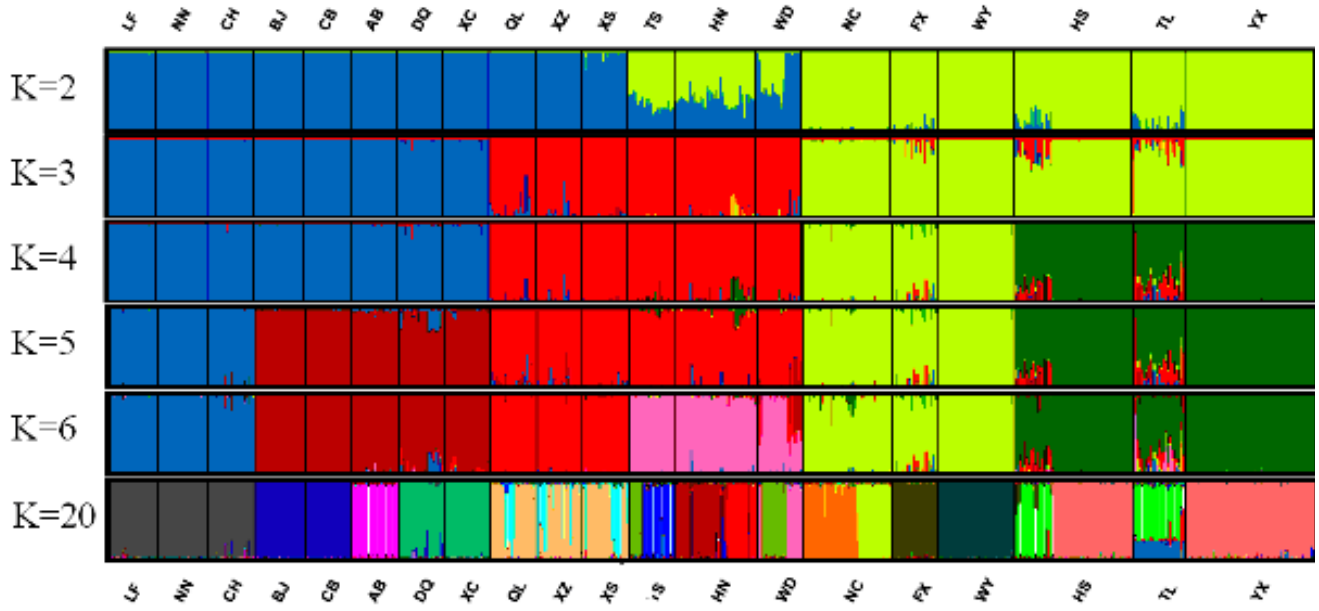

Figure 1. Population structure of the 20 *Apis cerana* populations by running Structure 100 times from K = 2 to 20.

ding inbreeding and outbreeding), selection and existence of 'null alleles'.

Genetic differentiation among populations

In this study, on average, the genetic differentiation (F_{ST}) among breeds was 42.3% (Table 4), a relative high value and extremely significant (P < 0.001), which indicated that there was a great differentiation among the 20 *A. cerana* populations. It was clear that about 42.3% of the total genetic variation corresponded to differences of populations and the remaining 58.7% was the result of differences among individuals. All loci contributed to this differentiation significantly.

The coefficient F_{IS}, 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, the mean of F_{IS} was as high as 0.612 (P < 0.001). In addition, 20 of the 21 loci (except A024d) showed significant deficit of heterozygotes. Two reasons may have contributed to the deficit of the heterozygotes for the nine loci: first, the locus may have been under selection (genetic hitchhiking effect) with some morphological or productive traits of selective interest and secondly, 'null alleles' may have been present.

Phylogenetic relationships among 20 *Apis cerana* populations

Environmental effects, historical process and life histories (mating system) may all, to some extent, constitute the genetic structure of populations. Geographical elements may owe to the close relationship for particular population pairs, for instance, Nanning bee and Fengxian bee. In the Neighbour-Joining tree, Nanning bee and Fengxian bee clustered together with 91.0 percent boostraps which indicated that they had a close genetic relationship. From geographical locations, Hunan province (Fengxian bee), is near to Guangxi (Nanning bee) and it was convenient for these populations to communicate with each other. Furthermore, the similar nectar plants, living customs between these two places made it easy to communicate with each other. The gene flow between these two breeds was very high (0.395). However, the result from Mantel's test failed to support a significant correlation between genetic and geographical pair wise distances for the whole dataset; all these results indicated that the geographical distribution was not a decisive factor to influence the genetic structure of *A. cerana* populations during their cultured history.

In the long history of animal domestication and breeding, in most main original areas with livestock relative sepa- rated regions without the convenient transportation, many local breeds were developed because of diversified geo- graphical conditions and lack of gene flow. For honeybee, the gene flow was more convenient by carrying queens from one area to other areas. So, relative separated regions could not prohibit the communication of honeybee populations. The results of this study also indicated that there was no significant correlation between the genetic and geographical pair wise distances among the 20 *A. cerana* populations.

Structure analysis indicated that populations from Eastern China formed one group which was confirmed by

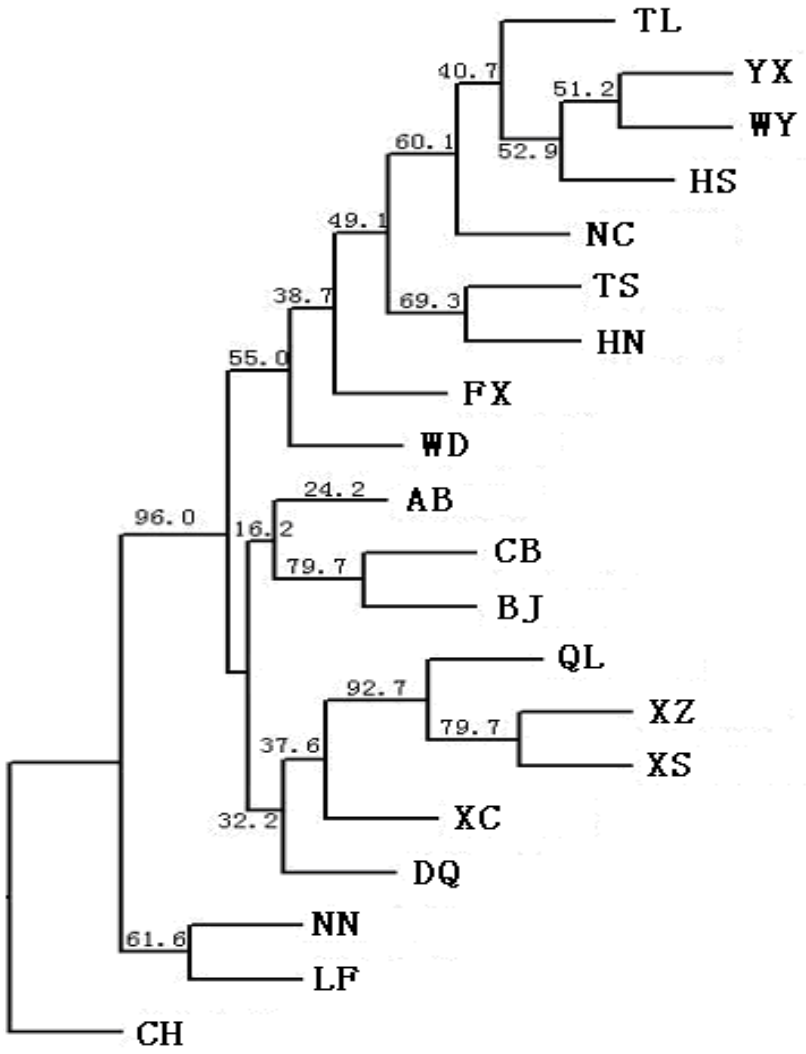

Figure 2. Dendogram of phylogenetic relationships among the 20 *Apis cerana* pupulations based on Reynolds' genetic distance, using neighbour-joining method; numbers at the nodes are percentage bootstrap values from 1000 replications with samples.

the results from NJ tree. Most of the Eastern China areas are low-altitude terrain and mainly plain and hilly areas, providing opportunity for frequent gene flow, so, the six populations formed the same genetic characteristics.

Several taxonomists demonstrated that Tibetan, Hainan and A-ba populations with distinct characteristics differed from *A. cerana* and may be a new sub-species according to the perspective of morphology and biochemical genetics (Gong and Zhang, 2000). Some researchers (Gong, 1980; Hong, 1984) took Diqing bee as Tibetan bee (*A. cerana himalaya*), because the two populations had the same morphological characteristics and distributions. However, the clustering result by molecular markers of this study did not support these conclusions.

In conclusion, all the 21 microsatellite loci in this study exhibited high or medium polymorphic and the average number of the alleles was 24.1430, which could provide enough effective and reliable information for the assessment of genetic diversity of the 20 *A. cerana* populations. At the 21 loci, the overall expected heterozygosity was 0.8378; Changbai bee had the lowest heterozygosity and Wuding bee the highest. *A. cerana* populations in Northern China showed a lower genetic variability and the priorities for the conservation of these precious populations should be conducted. There was a great differentiation among the 20 *A. cerana* populations, on average, the genetic differentiation (F_{ST}) among the breeds was 42.3%, a relative high value and extremely significant ($P < 0.001$); all loci contributed to this differentiation significantly. An overall significant deficit of heterozygotes (F_{IS}) was relatively high and the 20 loci showed significant deficit of heterozygotes. The clustering and structure results in this study concluded that Tibetan bee, Hainan bee and A-ba bee were all *A. cerana*

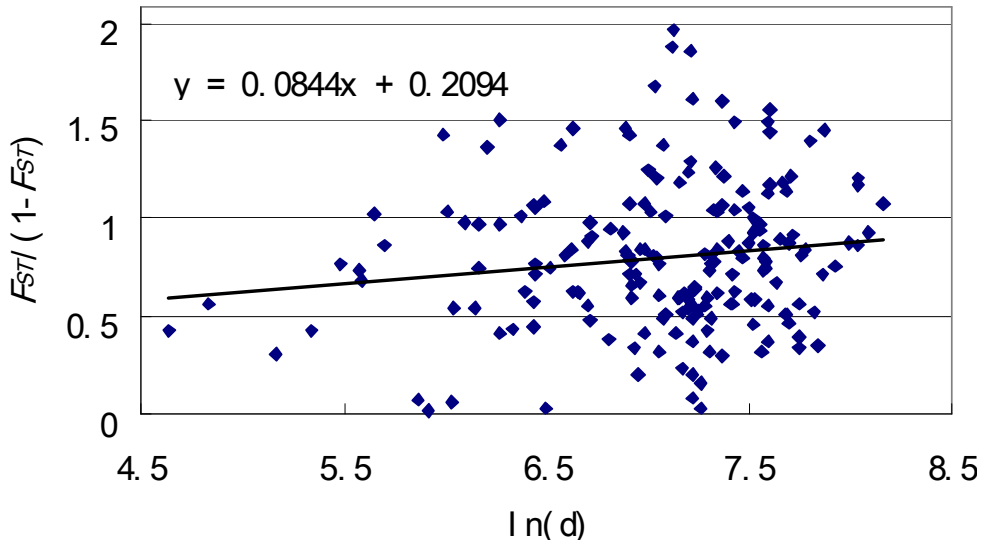

Figure 3. Plot of relationship between geographical distance ln(d) and pair wise *Fst*/(1-*Fst*) for the 20 *Apis cerena* populations.

populations; Diqing bee and Tibetan bee were the different populations, and they re *A. cerana* populations. There was no significant correlation between the genetic and geographical pairwise distances among the 20 *Apis cerana* populations. So, the geographical condition was only a reference when the program of *A. cerana* conservation was set up. The genetic distance should be served as the most important guide in determining priorities for conservation of *A. cerana* populations.

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