MORPHMETRIC ANALYSIS OF *APIS CERANA* POPULATIONS IN HUANGSHAN, CHINA

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Received 24 April 2013; accepted 25 October 2013

Abstract

Honey bees (*Apis cerana* Fabricius) were collected from 195 colonies at seven different localities spanning the main beekeeping areas in Huangshan. Morphometric methods were used to measure seven standard morphometric characters, and these bees were compared to samples from the Henan, Shandong, and Yunnan provinces. Principal component analysis of the total Huangshan database yielded two clusters: bees from Jinxian and Jixixian, and those from other localities. Within the latter cluster, discriminant and hierarchical cluster analyses revealed overlapping regional sub-clusters: bees from Huangshanqu, Qimenxian, Huizhouqu, and Shexian, and those from Yixian. Significant differences between the means of the three clusters were demonstrated using Wilks' lambda statistic. Morphocluster separation was related to altitude differences. Moreover, we noted some regions with high intercolonial variance, suggesting introgression among these defined honeybee populations.

Keywords: *Apis cerana*, Huangshan-China, morphology variation.

INTRODUCTION

Huangshan is located in the south end of the Anhui province of China (Wannan), between 117°02' - 118°55' east longitude and 29°24' - 30°24' north latitude. The city has a typical humid subtropical monsoon climate, and has considerably diverse flora and fauna, including honey bees. The honey bees (*Apis cerana* Fabricius) are an endemic population, well adapted to the agricultural and ecological environment in this area, with a particularly strong foraging instinct and resistance to parasitic mites and American foulbrood bee disease. *Apis cerana* F. has also been documented in Wannan (Wan D/XM01-19-87) according to ecological standards in the Anhui province. In 2001, this endemic honey bee population was listed in the directory of potential breeding stock for conservation. However, massive replacement of the

endemic honey bees has occurred, especially after the importation of *Apis mellifera*, with the endemic *Apis cerana* populations being almost completely replaced with imported stock in some regions (Yu et al., 2006). The number of honey bees in Wannan has severely decreased, with recent incomplete statistics showing an endemic honey bee population of only 15,000 in this area, which represents 40% of the total honey bees present in 1980s. Thus, the task of conserving the endemic honey bee population is becoming increasingly urgent (Yu et al., 2010). Conservation of the genetic diversity of domesticated plants has been well documented in Huangshan (Anna and Alan, 2006); however, few efforts have been made to preserve the genetic diversity of domesticated species, including honey bees. Discrimination between honey bee populations is important for beekeeping and for preserving honey bee biodiversity (Tofilski,

2008). Morphometry combining multivariate analysis is a common method for clarifying honey bee population structure. Multivariate morphometric studies of *A. cerana* have begun to clarify their population structure in many provinces in China, including Gansu (Tan and Qi, 2004), Hainan Island (Tan et al., 2004), Qinghai (Li et al., 2008), Sichuan (Luo and Tan, 2008), Henan (Wang and Shi, 2008), Shandong (Wang et al., 2008), and Yunnan (Tan et al., 2003). However, there remains little information about the morphological characters of these bees in the Anhui province. The only report of honey bee morphology in Anhui was performed by Yu et al. (2010), but this study could not adequately reveal the total honey bee population structure due to limited samples used for multivariate morphometric analysis.

The present study systematically investigated the morphological variation among *A. cerana* in Huangshan of the Anhui province in China, by collecting samples from seven different regions and using seven morphometric characteristics according to the method of Rutter et al. (1978). This work aimed to define the population structure of *A. cerana* in Huangshan, and to provide biogeographical information for conserving the genetic diversity of *A. cerana* in this area.

MATERIAL AND METHODS

The study was carried out on June in 2009. Honey bees were collected from 195 colonies from seven locations spanning the main beekeeping areas of Huangshan (Fig. 1), which lies in the south end of the Anhui province in China (Wannan). We obtained 24 colonies from Jingxian (JX) in the north part of Huangshan with an altitude of 286 m; 20 colonies were collected from Huangshanqu (HSQ) in northwest of Huangshan; 50 colonies were sampled from Jixixian (JXX) in north of Huangshan with an altitude of 262 m; 18 colonies were collected from Yixian (YX) in west of Huangshan with altitude of 298 m; 26 colonies each were obtained from Qimenxian (QMX) and Shexian (SX); and 31 colonies were sampled from Huizhouqu (HZQ) in the south of Huangshan.

Detailed collection data are given in Table 1. Each colony was represented by fifteen worker bees to use for morphological characteristics analysis.

The tested characteristics in this study included proboscis length (PL), right forewing length (FWL), right forewing width (FWW), forewing cubital a length (a), forewing cubital b length (b), tergite 3 width (T3), and tergite 4 width (T4). Honey bee samples were killed with hot water and preserved in 75% ethanol, and measurements were made according to the method of Ruttner et al. (1978). The main steps included dissection of the collected worker bee using forceps to separate the body parts (proboscis, right forewing, and tergite), which were then laid on a glass slide and examined with a calibrated micrometer. A digital camera was used to capture images of the separated body parts, which were then measured using a Photoshop program. All measurements are presented in mm.

The morphometric measuring program was used to calculate colony sample means and standard deviations, representing estimates for the colony. Univariate ANOVA was used to analyze the variation within and between localities. Differences among localities were confirmed by Bonferroni-adjusted post-hoc comparisons. Principal components analysis using colony mean data was performed to detect the presence of possible clusters of colonies among the scatter scores from a plotted plane graph of the first two high loading factors. Hierarchical cluster analysis using the principal component scores was used to determine the number of regional clusters. Discriminant analysis using the first component score was performed to classify the colonies and to determine the percentages of correctly classified colonies. We used Wilks' lambda statistic to test the significant difference between the multiple means of the characteristics entered into the discriminant functions, and Levene's F statistic to detect heterogeneity of variances (Johnson and Wichern, 2007). Lastly, the data for the Huangshan localities were compared to data for Henan (Wang and Shi, 2008), Shandong (Wang et al., 2008), and Yunnan (Tan et al., 2003) by hierarchical cluster analysis. All computations were performed using the SPSS 16 statistical package.

Fig. 1. Map of Huangshan indicating locations where bee samples were collected.

RESULTS

Table 1 lists the group means and standard deviations of colony means for the morphometric characteristics of the bees from the JX, HSQ, JXX, YX, QMX, SX, and HZQ regions of Huangshan in the Anhui province. Significant differences ($P < 0.001$) were detected for all seven characteristics. The bees from the YX locality exhibited extreme positions for four of the measures, showing the lowest values for proboscis length, forewing cubital a length, and forewing length and width. Differences were confirmed by Bonferroni-adjusted post-hoc comparisons, and all were significant ($P < 0.05$) with the following exceptions: proboscis length and forewing width in SX, forewing width and tergite 4 width in YX, and forewing cubital a length and tergite 4 width in QMX, tergite 4 width in JX, and proboscis length in HSQ and JXX.

Principal components analysis was carried out using the colony means of seven morphometric characteristics of 2925 worker honey bees from 195 colonies from seven localities. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.632, and Bartlett's test of sphericity was 203.98 ($P < 0.001$), demonstrating that the data were suitable for PCA. We extracted the principal factors with eigenvalues of 2.28 (PC1), 1.19 (PC2), and 1.08 (PC3). PC1 accounted for 32.5% of the total variation in the data, and was positively associated with labrum length, forewing length and width, and width of tergite 3 and tergite 4. PC2 accounted for 17.0% of the total variation in the data, and was positively associated with proboscis length. PC3 accounted for 15.4% of the total variation in the data, and was negatively associated with length of forewing cubital a. Together, these factors accounted for about 64.9% of the total variance in the data. Figure 2 shows the plots of sample factor scores on the two principal component

Table 1.

Means and standard deviations and one-way analyses of seven morphological characteristics of honey bees from seven different regions of Huangshan

Characteristics	JX	HSQ	JXX	YX	QMX	SX	HZQ
PL	5.338 ± 0.365 ^A	5.153 ± 0.239 ^{AB}	5.121 ± 0.431 ^{AB}	4.631 ± 0.632 ^C	4.989 ± 0.533 ^{AB}	4.886 ± 0.641 ^{BC}	5.178 ± 0.332 ^{AB}
FWL	PL8.655 ± 0.474 ^{BC}	8.574 ± 0.418 ^{BC}	8.700 ± 0.467 ^B	8.342 ± 0.365 ^C	9.336 ± 0.376 ^A	8.740 ± 0.349 ^B	9.164 ± 0.482 ^A
FWW	2.916 ± 0.053 ^{CB}	2.995 ± 0.092 ^A	2.903 ± 0.061 ^C	2.903 ± 0.105 ^C	3.001 ± 0.124 ^A	2.983 ± 0.141 ^{AB}	2.981 ± 0.108 ^{AB}
a	0.553 ± 0.060 ^A	0.556 ± 0.049 ^A	0.544 ± 0.050 ^A	0.491 ± 0.050 ^B	0.537 ± 0.045 ^A	0.544 ± 0.051 ^A	0.574 ± 0.059 ^A
b	0.213 ± 0.029 ^A	0.157 ± 0.015 ^D	0.200 ± 0.035 ^{AB}	0.183 ± 0.028 ^{BC}	0.173 ± 0.020 ^{CD}	0.172 ± 0.022 ^{CD}	0.192 ± 0.026 ^{BC}
T3	1.809 ± 0.088 ^B	1.890 ± 0.086 ^A	1.789 ± 0.076 ^B	1.933 ± 0.185 ^A	1.903 ± 0.052 ^A	1.960 ± 0.104 ^A	1.937 ± 0.070 ^A
T4	1.791 ± 0.072 ^{AB}	1.835 ± 0.089 ^A	1.743 ± 0.121 ^B	1.787 ± 0.112 ^{AB}	1.812 ± 0.047 ^{AB}	1.856 ± 0.086 ^A	1.851 ± 0.122 ^A

The superscript letters A, B, and C are used to indicate that means for the same characteristics followed by different letters among locations are significantly different ($P < 0.01$) according to one-way analysis followed by Duncan's multiple range tests.

Fig. 2. Characterization of *A. cerana* colony samples from seven localities of Huangshan. Axes show sample means factor 1 scores (x-axis) plotted against factor 2 scores (y-axis).

Fig. 3. Hierarchical clustering dendrogram for *A. cerana* in seven different locations of Huangshan for seven examined characteristics

Table 2.

Classification matrix between three Huangshan honey bee groups:
group 1 includes colonies from JX and JXX; group 2 includes colonies from HSQ, SX, QMX, and HZQ; and group 3 includes colonies from YX

Groups		Predicted Group Membership			Total
		Group 1	Group 2	Group 3	
Count	1	62	12	0	74
	2	8	79	15	102
	3	0	4	14	18
%	1	83.8	16.2	0	100
	2	7.8	77.5	14.7	100
	3	0	22.2	77.8	100

Table 3.

Means and standard deviations (SD) of seven morphometric characteristic measurements (in mm) for three morphoclusters of *A. cerana* in Huangshan:
group 1 includes colonies from JX and JXX; group 2 includes colonies from HSQ, SX, QMX and HZQ; and group 3 includes YX

Character	Group1 n = 74	Group 2 n = 102	Group 3 n = 18
LL	5.191 ± 0.421	5.051 ± 0.478	4.631 ± 0.632
FWL	8.686 ± 0.467	8.999 ± 0.494	8.342 ± 0.365
FWW	2.907 ± 0.059	2.990 ± 0.117	2.903 ± 0.105
A	0.547 ± 0.053	0.553 ± 0.053	0.491 ± 0.050
B	0.204 ± 0.033	0.176 ± 0.024	0.183 ± 0.028
T3	1.796 ± 0.080	1.925 ± 0.083	1.933 ± 0.185
T4	1.759 ± 0.110	1.839 ± 0.092	1.787 ± 0.112

n - number of colonies

axes, revealing two indistinct morphoclusters. Honey bees from JX and JXX formed one cluster in the upper first and second quadrants of the plot, while honey bees from other localities formed another cluster.

To further determine the number of regional clusters within the second cluster, hierarchical cluster analysis was conducted using the locality mean canonical scores obtained from the group factors. Figure 3 shows a dendrogram

of three main clusters obtained on the linkage distance level of 10. Regional cluster 1 (JX and JXX) first linked to the colonies from YX (cluster 2), followed by those from HSQ, SX QMX, HZQ, which formed the third regional cluster. The discriminant analysis results were as follows: from JX and JXX, 83.8% of colonies ($n = 74$) were correctly classified into group 1, while 12 colonies were misclassified into group 2; from HSQ, SX, QMX, and HZQ, 77.5% colonies ($n = 102$) were correctly classified into group 2, while 8 were misclassified into group 1, and 15 into group 3; from YX, 77.8% colonies ($n = 18$) were correctly classified into group 3 and 4 were misclassified into group 2 (Tab. 2).

Wilk's lambda value and the F statistic revealed a remarkably significant difference among the means of morphometric characteristics for the three groups entered into the discriminant functions ($\Delta = 0.326$; $F_{14,370} = 19.832$; $P < 0.0001$). Table 3 shows the group means and standard deviations of morphometric characteristics. The first principal components coefficients of the morphometric characteristics were used to compute the factor scores of colonies at each locality, and the variances of these scores were tested for homogeneity of intercolonial variance at each locality. Significant differences were found among the means of the groups (Levene's test $F_{6,188} = 7.949$; $P < 0.0001$). Multiple comparisons further indicated remarkably significant differences ($P < 0.01$) between cluster 1 and cluster 3 in PL, FWL, a, b, and T3; between cluster 1 and cluster 2 in FWL, FWW, b, T3, and T4; and between cluster 2 and cluster 3 in PL, FWL, FWW, and a.

We also found significant negative correlations between the honey bee size and the altitudes of the localities at which they were

collected. The correlations between forewing length and altitude using all seven localities was remarkably significant ($r = -0.948$; $P < 0.01$), and a significant correlation was also found between the forewing width and attitude ($r = -0.763$; $P < 0.05$). The data for the Huangshan localities were compared to data for the adjacent three provinces in China by hierarchical cluster analysis. Figure 3 shows that the honey bees from Huangshan firstly linked to the bees from Shandong, and then linked to colonies of Henan. Honey bees from Yunnan formed a separate group.

DISCUSSION

Multivariate morphometric analysis of Huangshan honey bees revealed a high degree of variability in the proboscis length, and forewing length and width. Proboscis lengths of honey bees from clusters 1 and 2 were significantly different from those of cluster 3 (YX region). Among the investigated areas, YX was the locality with the highest altitude, suggesting that geographical variability contributes to proboscis variability. This is consistent with the report of Marghitas et al. (2008), which showed that proboscis length is a very important characteristic for indicating geographical variability. Proboscis length variations have also been shown to be related to the environmental resources in Huangshan (Souza et al., 2002), and proboscis length is known to influence the choice of nectar resources in bumblebees (Arbulo et al., 2011). Moreover, the morphometric characteristics associated with forewing size were significantly negatively correlated to altitude. This result is opposite to those reported by Tan et al. (2003), which may be due

Fig. 4. Characterization of *A. cerana* from Huangshan and from the adjacent regions of Henan, Shandong and Yunnan.

to the low altitude of all regions where honey bees were collected in their study.

Our present discriminant analysis of honey bees from 195 colonies from seven localities in Huangshan revealed the existence of three statistically separable morphometric groups. One group was further divided into two sub-groups. Such separation was associated with geographical position, as HZQ and QMX are located in the northeast of Huangshan with similar altitudes of 153 and 129 m, respectively, while HSQ and SX are located in the southwest of Huangshan with respective altitudes of 244 and 235 m. Morphometrically, the honey bees from western, low altitude parts of the city (cluster 2), had the longest forewing width (2.990 mm) and length (8.999 mm), indicating their higher flight power and ability to collect more pollen and nectar (Mostajeran et al., 2006). The mean values of the morphometric characteristics of this group were very similar to those previously reported for *A. cerana* in the Henan province (Wang and Shi, 2008). Honey bees of cluster 3 were from YX, which has an altitude of 298 m. A very small honey bee group was detected in this locality, which may be attributed to the long-term rainfall in this area and the lower annual average temperature of 15.8°C.

A hierarchical cluster analysis among Huangshan and other provinces in China revealed that colonies from Huangshan were grouped with Henan and Shandong. These three are adjacent to each other and lie at similar low altitudes. On the other hand, another separate branch was formed by bee samples from Yunnan, which is far away from the other three localities and which is located at a high altitude. These findings demonstrated that the morphological characteristics were associated with geography.

The presently examined honey bee clusters exhibited high intercolonial variance, especially in wing size and proboscis length. These findings revealed probable introgression among defined honey bees groups. This could be attributed mainly to swarming and migration, purchase of queen, and transhumance, which may greatly contribute to gene flow among the honey bee clusters.

CONCLUSIONS

The present multivariate analyses detected great morphological diversity among the examined honey bee populations. All of the populations from the seven different regions were divided into three main clusters: JX; JXX, HSQ, and QMX; and HZQ and SX.

ACKNOWLEDGEMENTS

This work was supported by grants from the Earmarked Fund for Modern Agro-industry Technology Research System (CARS-45-KXJ9), National Natural Science Foundation of China (No. 31302039), and Natural Science Foundation of Anhui Province (03041202, 070411016). We are grateful to Wu Chengwu, the director of Bureau of Animal Husbandry of Huangshan in the Anhui Province, and to the Five Bee Home Limited Company to help with bee collecting. We also thank Ji Ting and Yin Ling from Yangzhou University for their kind help with this research.

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