



HAL
open science

Multivariate morphometric analysis of the *Apis cerana* of China

Ken Tan, H. Randall Hepburn, Sarah E. Radloff, Stefan Fuchs, Xian Fan, Lianjiang Zhang, Mingxian Yang

► **To cite this version:**

Ken Tan, H. Randall Hepburn, Sarah E. Radloff, Stefan Fuchs, Xian Fan, et al.. Multivariate morphometric analysis of the *Apis cerana* of China. *Apidologie*, 2008, 39 (3), pp.343-353. hal-00891914

HAL Id: hal-00891914

<https://hal.science/hal-00891914>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Multivariate morphometric analysis of the *Apis cerana* of China*

Ken TAN^{1,5}, H. Randall HePBURN^{2,5}, Sarah E. RADLOFF³, Stefan FUCHS⁴, Xian FAN⁵,
Lianjiang ZHANG⁵, Mingxian YANG⁵

¹ Xishuangbanna Tropical Botanical Garden, Chinese Academy of Science, Kunming 650223, Yunnan Province, China

² Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa

³ Department of Statistics, Rhodes University, Grahamstown 6140, South Africa

⁴ Institut für Bienenkunde (Polytechnische Gesellschaft), Fachbereich Biowissenschaften der J.-W. Goethe Universität, Frankfurt am Main, 61440 Oberursel, Germany

⁵ Eastern Bee Research Institute of Yunnan Agricultural University, Heilongtan, Kunming, 650201, China

Received 25 January 2007 – Revised 8 January 2008 – Accepted 29 January 2008

Abstract – Multivariate statistical analyses of the morphometric characters of worker bees of *Apis cerana* were collected from 188 colonies at 68 localities throughout China with a sampling resolution of 1 locality/50 000 km². Principal components plots revealed one morphocluster. By introducing local labeling it became clear that bees from different localities form overlapping regional clusters: 1. bees from Jinlin, Liaoning, Beijing and northern Shanxi provinces; 2. larger bees from southern Gansu and northern and central Sichuan; 3. smaller bees from southern Yunnan, Guangdong, Guangxi, Hong Kong and Hainan; 4. bees from the rest of China. Hierarchical cluster analysis using data for the China regional groups 1 to 4 and adjacent countries yielded a dendrogram of two main clusters. Colonies from Japan, Korea and Russia were linked with those from China regional groups 1, 2 and 4; colonies from northern Vietnam, north eastern India, Thailand and Myanmar were linked with those from China regional group 3. *A. cerana* populations are continuous and panmictic, and morphometric structuring is indistinct.

morphometric analysis / *Apis cerana* / China

1. INTRODUCTION

Several univariate biometric studies of morphological characters of *Apis cerana* Fabricius of China have appeared through the years (Kellogg, 1930 et seq.; Gong, 1978, 1983a, b; Liu et al., 1982; Zhuang, 1982; Xun, 1984; Kuang, 1986, 1988; Kuang and Li, 1988), but their methodological differences preclude any synthesis of them. However, Yang and colleagues produced a lengthy series of studies Yang (1982 et seq.) from which five proposed

biometric groups and several ecotypes eventually emerged (Yang, 2001). Peng et al. (1989) and Zhen-Ming et al. (1992) subsequently endorsed these conclusions. But, unfortunately, neither the original raw data nor the statistical methods of analysis were included in the original publications and the original honeybee specimens no longer exist (Yang, pers. com.). Some of these publications formally described several new subspecies of *A. cerana*: *A. c. maerkang*, *A. c. cathayca*, *A. c. bijjieca*, *A. c. twolareca*, *A. c. fantsum*, *A. c. pekinga*, *A. c. kweiyanga*, *A. c. shankianga*, *A. c. abansensis*, and *A. c. abansis*; but, none of these names have ever been cited in any of the honeybee literature written in European

Corresponding author: S.E. Radloff,
s.radloff@ru.ac.za

* Manuscript editor: Walter S. Sheppard

languages. Aside from the inherent difficulties of formal scientific names for subspecies of Chinese *A. cerana*, there are also different views about races/subspecies for which common names (in Chinese or English) are used in China.

They include highland and lowland populations on Hainan Island; an Eastern Chinese honeybee with Guangxi and Guangdong, Hunan, Yungui Plateau, North China, and Changbai Mountains ecotypes; a South Yunnan group; and a South Tibetan bee. In addition, the Chinese literature refers to several populations of bees grouped differently and based on a combination of some morphometric data, physiology, pigmentation, cell size, low temperature tolerance and foraging (Hou and Hou, 1983; Yang, 2001; Zhen-Ming et al., 1992). In a recent series of a multivariate analyses, Tan and Zhang (2001 et seq.) morphometrically defined two major morphoclusters of *A. cerana* in China each being correlated with a specific ecological zone: (1) the southern tropical seasonal rain forest region and (2) another cluster containing bees from the temperate deciduous broad-leaved forest region, the subtropical evergreen broad-leaved forest zone, and the high, cold meadow and steppe region, which can be refined into three subclusters. Although that study included material from 19 widely spread localities, given the enormity of China, these data represented a sampling penetration of only 1 locality/250 000 km² of non-desert land area. In parallel with related studies on *A. cerana*, we have greatly extended the sampling penetration nearly five-fold to cover all China with the exception of the Xizang Zizhiqu Autonomous Region (= Tibet). Because morphocluster resolution in multivariate analyses is enhanced with increasing sampling distance (Radloff and Hepburn, 1998), analysis of this new material provides more refined confidence levels in defining population structure and geographical variation for the *A. cerana* of China.

2. MATERIALS AND METHODS

2.1. Honeybees

The worker honeybee specimens used for analysis derive from several different sources. (1) New

material has been collected throughout China from the provinces of Beijing, Gansu, Guangdong, Guangxi, Henan, Anhui, Hubei, Hujian, Hunan, Jiangxi, Jilin, Ningxia, Shanxi, Sichuan and Hainan island (Kunming database, 125 colonies) and from Hong Kong, Beijing, Shandong, Chengdu and Yaan (Grahamstown database, 37 colonies). (2) Data from recent work on the *A. cerana* of Yunnan Province, China (Radloff and Hepburn, 2002; Tan et al., 2002a, b; 2003a, b) are included here as are those previously analyzed by Ruttner (1988). The geographical origins of worker honeybees of *A. cerana* from 188 colonies at 68 localities used in the current analysis are listed in Table I and shown in Figure 1. The honeybee samples represent different climatic or vegetation regions, and ranged in altitude from 8 m to 2820 m (Fig. 1). The bees were collected from natural nests or semi-managed hives as well as logs or wall cavities. Movable-frame beehives have been introduced in some areas only recently, and migration of beehives is uncommon, so that the bees were authentic samples of the wild populations.

2.2. Measurements

At each locality, 1–10 colonies were sampled and each sample contained 15–30 worker bees, which were killed and preserved in 75% ethanol. From each sample fifteen worker bees were dissected for morphometric analysis and measured according to the methods described by Ruttner et al. (1978) and Ruttner (1988). Of the 41 morphometric characters listed in Ruttner et al. (1978) 38 were measured, excluding length of proboscis (No. 4) and cubital veins distances (No. 19 and 20), resulting in 16 size characters, 11 wing angles, 7 color characters, 3 hair characters, and hamuli. Colony sample means and standard deviations were computed for each character from the samples by the morphometric measuring program, representing estimates for the colony. Measurements and color scaling were performed using a stereomicroscope and a computer-aided measuring system based on a video system and measuring program (Bee2, © Meixner, 1994). The Kunming and Oberursel databases included measurements of thirty-five morphological characters from Ruttner (1988) (proboscis length omitted). Twelve morphological characters were common to the Kunming/Oberursel and Grahamstown databases. Their Ruttner (1988) numbers are given in round brackets as follows: length of femur (5), length of tibia

Table I. Distribution of localities, co-ordinates, altitude (m), and number of colonies (n) sampled in China. Columns G3.1 and G3.2 indicate the morphometric regional clusters in which the colonies were classified with cluster analyses using 26 and 12 morphometric characters, respectively. ? indicates the localities where the colonies are unclassified.

Location	Co-ordinates	Altitude	n	G3.1	G3.2
1. Jingyu (Jilin)	42.23N 126.48E	530	3	1	1
2. Linjiang (Jilin)	41.48N 126.54E	309	3	1	1
3. Xinbin (Liaoning)	41.43N 125.02E	323	3	1	1
4. Jiangyuan (Jilin)	41.34N 126.01E	560	3	1	1
5. Changbeishan (Jilin)	41.24N 128.11E	687	3	1	1
6. Ji an (Jilin)	40.55N 125.53E	780	2	1	1
7. Xiangshan (Beijing)	39.58N 116.12E	76	4	1	1
8. Beijing	39.55N 116.23E	63	8	1	1
9. Jingbian (Shaanxi)	37.36N 108.45E	1370	2	1	1
10. Suide (Shaanxi)	37.29N 110.15E	885	1	1	1
11. Qingjian (Shaanxi)	37.15N 110.15E	1200	2	1	1
12. Qingcheng (Ganshu)	36.04N 107.52E	1258	2	?	?
13. Guyuanxi (Ningxia)	36.04N 106.16E	1671	3	?	?
14. Taiqian (Henan)	36.00N 115.45E	460	3	4	4
15. Mengjin (Shandong)	35.42N 117.55E	180	5		4/1
16. Pingliang (Ganshu)	35.32N 106.41E	1355	3	?	?
17. Dingxili (Ganshu)	35.13N 103.57E	2024	3	2	2
18. Mengjin (Henan)	34.57N 112.30E	180	3	4	4
19. Tianshui (Ganshu)	34.48N 105.32E	1200	2	2	2
20. Shanxian (Henan)	34.45N 111.18E	360	4	4	4
21. Lingtan (Ganshu)	34.34N 103.50E	1154	2	2	2
22. Mingxian (Ganshu)	34.33N 103.51E	2439	7	2	2
23. Ruyang (Henan)	34.09N 112.27E	750	3	4	4
24. Lushi (Henan)	34.02N 111.01E	100	3	4	4
25. Yuwaqu (Sichuan)	33.55N 103.28E		3	2	2
26. Jiaoqu (Sichuan)	33.37N 104.39E		3	2	2
27. Zhumadia (Henan)	33.30N 113.45E	72	1	4	4
28. Yonglezheng (Sichuan)	33.30N 104.26E		2	2	2
29. Wujiaoxiang (Sichuan)	33.15N 104.22E		3	2	2
30. Jiuzhaigon (Sichuan)	32.36N 103.54E	1200	4	2	2
31. Xinyangnan (Henan)	32.05N 114.07E	1100	3	4	4
32. Shengnongjia (Hubei)	31.43N 110.43E	1166	6	4	4
33. Chengdhu (Sichuan)	30.40N 104.04E	529	5		4/1
34. Huangshan (Anhui)	30.10N 118.21E	300	2	4	4
35. Anyue (Sichuan)	30.08N 105.37E	325	2	4	4
36. Ya-an (Sichuan)	29.58N 103.04E	588	10		2
37. Chongqin (Sichuan)	29.15N 107.12E	608	3	4	4
38. Pingjian (Hunan)	28.53N 113.56E	57	1	4	4
39. Hengdong (Hunan)	27.04N 112.57E	34	4	4	4
40. Lijiang (Yunnan)	26.52N 100.13E	2680	2	4	4
41. Wugan (Hunan)	26.48N 110.51E	420	1	4	4
42. Yuxian (Hunan)	26.31N 113.31E	280	1	4	4
43. Huzhou (Hujian)	26.15N 119.20E	168	1	4	4
44. Sayinpan (Yunnan)	26.01N 102.31E	2279	2	4	4
45. Lushui (Yunnan)	25.57N 98.49E	1950	2	4	4
46. Huize (Yunnan)	25.55N 103.26E	1550	1	3	3
47. Binchuan (Yunnan)	25.51N 100.33E	1690	2	4	4
48. Yuangbi (Yunnan)	25.39N 99.56E	1810	2	4	4

Table I. Continued.

Location	Co-ordinates	Altitude	n	G3.1	G3.2
49. Huichang (Jiangxi)	25.36N 115.47E	460	3	4	4
50. Jianghua (Hunan)	25.01N 111.45E	305	1	4	4
51. Pingle (Guangxi)	24.45N 107.58E		2	4	4
52. Zhenkang (Yunnan)	24.00N 98.55E	868	2	3	3
53. Laibin (Guangxi)	23.41N 109.16E	180	1	3	3
54. Kaiyuan (Yunnan)	23.41N 102.05E	1148	2	3	3
55. Yuanjian (Yunnan)	23.35N 101.59E	1500	1	3	3
56. Caoba (Yunnan)	23.32N 103.23E	1293	2	3	3
57. Guangzhou (Guangdong)	23.11N 112.48E	100	1	3	3
58. Nanhai (Guangdong)	23.01N 113.08E	8	2	3	3
59. Pingbian (Yunnan)	22.50N 103.43E	1400	2	3	3
60. Simao (Yunnan)	22.43N 100.56E	1100	2	3	3
61. Hekou (Yunnan)	22.30N 103.57E	122	2	3	3
62. Hong Kong	22.16N 114.09E	88	12		4/3
63. Jinghong (Yunnan)	21.55N 100.52E	600	2	3	3
64. Yongxing (Hainan)	19.53N 110.15E	150	1	3	3
65. Dingan (Hainan)	19.41N 110.19E	200	1	3	3
66. Wenchang (Hainan)	19.37N 110.45E	80	1	3	3
67. Qiongzong (Hainan)	19.02N 109.50E	260	1	3	3
68. Jianfeng (Hainan)	18.42N 108.48E	100	2	3	3

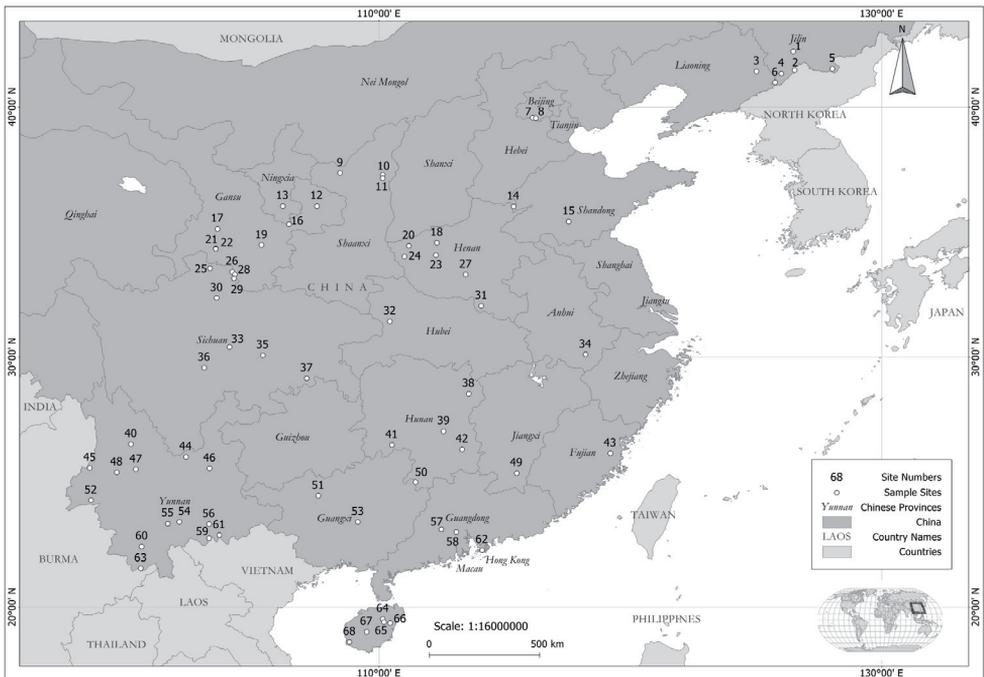


Figure 1. Geographical localities in China at which the *Apis cerana* used in these analyses were collected. Names of the localities and their co-ordinates are given in Table I.

(6), metatarsus length (7), tergite 3, longitudinal (9), tergite 4, longitudinal (10), sternite 3, longitudinal (11), wax plate of sternite 3, longitudinal (12), sternite 6, longitudinal (15), forewing, longitudinal (17), wing angle B4 (22), wing angle D7 (23) and wing angle G18 (25).

2.3. Data analysis

Multivariate statistical analysis of the data included principal components analysis to identify possible morphoclusters, k-means non-hierarchical cluster analysis to identify the colonies within each morphocluster, linear discriminant analysis to determine the percentages of correct classification of colonies in each morphocluster, analysis of variance, Wilks' lambda statistic for testing significant differences between the multiple means of the characters entered into the discriminant functions and Levene's F statistic procedure for testing heterogeneity of variances and lastly hierarchical cluster analysis to identify homogeneous morphoclusters for *A. cerana* in China and adjacent countries based on 12 common morphometric characters (Johnson and Wichern, 2002; Rao, 1998). The analysis was carried out in three stages: (1) Kunming and Oberursel databases were analysed using 26 characters; (2) Kunming/Oberursel and Grahamstown databases using 12 common characters; (3) Kunming/Oberursel, Grahamstown and reference samples from adjacent countries. All statistical analyses were performed using Statistica[®](StatSoft, 2006).

3. RESULTS

3.1. Analysis on Kunming and Oberursel databases

Principal components analysis was carried out on the Kunming and Oberursel morphometric databases of 151 colonies of *A. cerana* collected throughout China using 26 morphometric characters of worker honeybees (Ruttner numbers, (1) to (3), (5) to (12), (15) to (18) and (21) to (31)). Six principal components with eigenvalues greater than one were isolated and accounted for a total of 72.9% of the variation in the data. PC 1, size-related characters (1), (2), (5) to (12), (15), (17) and (18) with

component loadings between 0.62 and 0.93 accounting for 35.1% of the variation; PC 2, size-related character (16), and angles of venation B4 (22) and E9 (24) with component loadings between 0.55 and 0.73, accounting for 12.0% of the variation; PC 3, angles of venation J16 (27) and N23 (30) with component loadings 0.70 and 0.85, respectively, accounted for 8.9% of the variation. The other 8 characters had component loadings less than 0.50.

PC plots using both the first and second PC scores, and the first and third PC scores suggested one morphocluster. Further substructuring of the morphocluster could not initially be derived from the PC plots; because only by first introducing local labeling does it become clear that bees from different localities form overlapping regional clusters: (1) bees from Jinlin, Liaoning, Beijing and northern Shanxi provinces; (2) larger bees from southern Ganshu and northern and central Sichuan (larger Aba bees); (3) smaller bees from southern Yunnan, Guangdong, Guangxi, Hong Kong and Hainan and (4) bees from the rest of China (Fig. 2).

Having established four regional clusters, a k-means cluster analysis was used to group the colonies (Tab. I, column G3.1). Lastly, a stepwise discriminant analysis using the four groups entered the following 16 characters in order of discriminatory powers: (18), (1), (9), (16), (8), (6), J16 (27), E9 (24), B4 (22), (2), (17), (11), (15), (5), (10), and (7). The results classified 96.3% of colonies ($n = 27$) from north eastern China (Jinlin, Liaoning, Beijing and northern Shanxi provinces) correctly into group 1 and misclassified one colony into group 4; 93.1% of the colonies ($n = 29$) from southern Ganshu and northern and central Sichuan into group 2 (larger bees) but misclassified two colonies into group 4; 96.2% of the colonies ($n = 26$) from southern Yunnan, Guangdong, Guangxi and Hainan into group 3 (smaller bees) and misclassified one colony into group 4 and 91.3% of the colonies ($n = 69$) from central, eastern China into group 4 and misclassified three, two and one colonies into groups 1, 3 and 2, respectively. To test the equality of the group means for the characters used in the discriminant function, Wilks'

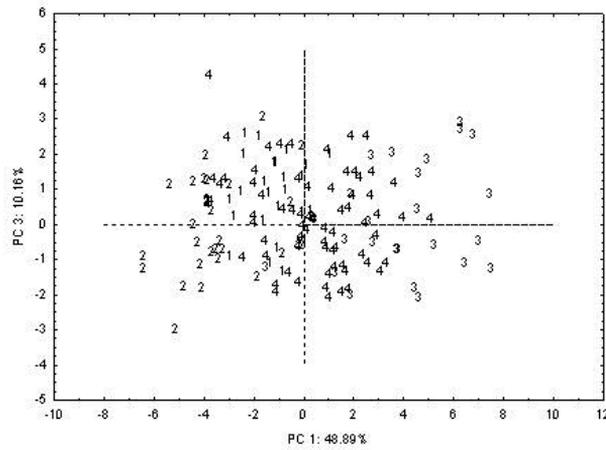


Figure 2. Principal components plot of the morphocluster of *A. cerana* in China using 26 morphometric characters (Number of colonies n = 151). Numbers indicate colonies from regional clusters: 1 = colonies from Jinlin, Liaoning, Beijing and northern Shanxi provinces; 2 = colonies from southern Ganshu and northern and central Sichuan (larger *Aba* bees); 3 = colonies from southern Yunnan, Guangdong, Guangxi, Hong Kong and Hainan and 4 = colonies from the rest of China.

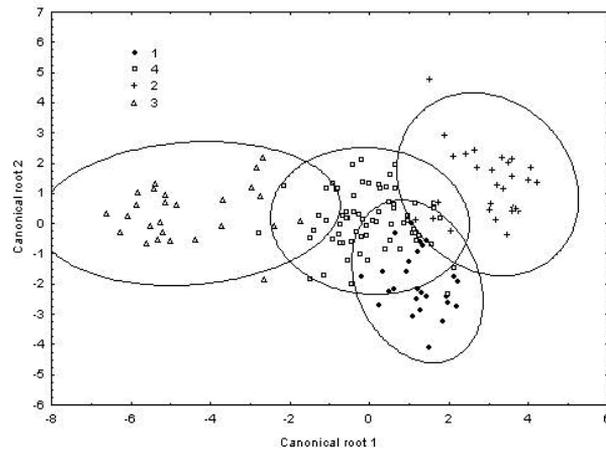


Figure 3. Discriminant analysis plot using the colony means of the morphometric data (Ruttner numbers, (18), (1), (9), (16), (8), (6), J16 (27), E9 (24), B4 (22), (2), (17), (11), (15), (5), (10), and (7)). Regional clusters: 1 = Jinlin, Liaoning, Beijing and northern Shanxi provinces; 2 = southern Ganshu and northern and central Sichuan; 3 = southern Yunnan, Guangdong, Guangxi, Hong Kong and Hainan; 4 = the rest of China. Confidence ellipses are drawn at the 95% level.

lambda approximated by the F statistic was determined. A significant difference between the means of the four groups was established ($\Lambda = 0.0498, F_{48,393} = 14.3, P < 0.0001$).

Canonical 1 and 2 scores plot showed that groups 1 and 3 and groups 2 and 3 are disjoint (Fig. 3, confidence ellipses at 95%).

The squared Mahalanobis distances between the centroids of the groups are given in Table II. The colony means and standard deviations of 28 morphometric characters ((1), (2), (5) to (18), angles of venation (21) to (31) and hamuli) averaged for regional groups are shown in Table III.

Table II. Mahalanobis squared distances between centroids of the four morphometric regional groups: 1 = Jinlin, Liaoning, Beijing and northern Shanxi provinces; 2 = southern Ganshu and northern and central Sichuan; 3 = southern Yunnan, Guangdong, Guangxi, Hong Kong and Hainan; 4 = the rest of China.

	Regional Groups			
	1	2	3	4
1	–	12.1	38.0	7.3
2	12.1	–	54.8	11.6
3	38.0	54.8	–	22.6
4	7.3	11.6	22.6	–

3.2. Analysis on Kunming/Oberursel and Grahamstown databases

Principal components analysis was carried out on the Kunming/Oberursel database together with the Grahamstown database of *A. cerana* colonies from Hong Kong, Beijing, Shandong, Chengdu and Yaan using 12 common morphometric characters of worker honeybees ($n = 188$ colonies; Ruttner numbers, (5), (6), (7), (9), (10), (11), (12), (15), (17), B4 (22), D7 (23) and G18 (25)). Three principal components with eigenvalues greater than one were isolated and accounted for a total of 74.3% of the variation in the data. PC 1, size-related characters (5) to (7), (9) to (12), (15) and (17) with component loadings between 0.73 and 0.93 accounting for 53.1% of the variation; PC 2, angles of venation B4 (22) and G18 (25) with component loadings 0.71 and 0.54, respectively, accounting for 11.8% of the variation; PC 3, angle of venation D7 (23) with component loading 0.77 accounting for 9.4% of the variation.

PC plots using both the first and second PC scores, and the first and third PC scores again revealed four similar regional groups (Fig. 4). A stepwise discriminant analysis using the four groups entered all 12 characters into the discrimination function. All five colonies from Beijing were correctly classified into group 1; all five colonies from Chengdu and ten colonies from Yaan were correctly classified into group 2 (larger bees); two of the five colonies from Shandong were correctly classified into group 4 and three were misclassified

into group 1, and ten of the twelve colonies from Hong Kong were correctly classified into group 3 (smaller bees) and two were misclassified into group 4 (Tab. I, column G3.2). A significant difference between the means of the four groups was again established ($\Lambda = 0.0876$, $F_{36,511} = 18.2$, $P < 0.0001$).

3.3. Analysis on Kunming/Oberursel, Grahamstown and adjacent countries databases

An hierarchical cluster analysis (Quinn and Keough, 2002) using the mean values of 12 common characters for the China regional groups 1 to 4 and adjacent countries yielded a dendrogram of two main clusters (Fig. 5). Phenetically, colonies from Japan and Korea were first linked with those from China regional groups 1 and 4, followed by those from regional group 2, and finally those from Russia; colonies from northern Vietnam and north eastern India were first linked with those from China regional group 3, followed by colonies from Thailand and Myanmar.

4. DISCUSSION

The initial mesoscale morphometric analysis of the honeybees for the whole of China suggested one morphocenter (Fig. 2). However with local labeling these could be refined into four partially overlapping clusters which were confirmed by discriminant analysis (Fig. 3). Because the three different databases contained variable numbers of available characters, separate analyses were performed first on the Kunming/Oberursel database with more shared characters and then these data were combined with those of Grahamstown with fewer shared characters. Nonetheless, even with a collectively reduced total database, the same cluster discrimination was obtained in both analyses (Figs. 2–4).

That the four groups of bees formed overlapping clusters points to the fact that, despite the immense area which constitutes China, the *A. cerana* populations are continuous and panmictic, showing only moderate and instinct geographical structuring. Indeed the average of

Table III. Means and standard deviations (sd) of 28 morphometric character measurements (mm or angles in degrees) of *A. cerana* worker honeybees from four regional groups: 1 = Jinlin, Liaoning, Beijing and northern Shanxi provinces; 2 = southern Ganshu and northern and central Sichuan; 3 = southern Yunnan, Guangdon, Guangxi, Hong Kong and Hainan; 4 = the rest of China. n = total number of colonies in each region.

character	Regional Groups							
	1 (n = 32)		2 (n = 44)		3 (n = 38)		4 (n = 74)	
	mean	sd	mean	sd	mean	sd	mean	sd
(1)	0.36	0.03	0.44	0.04	0.22	0.05	0.37	0.06
(2)	0.42	0.04	0.41	0.04	0.34	0.05	0.37	0.05
(5)	2.48	0.04	2.55	0.06	2.41	0.06	2.46	0.06
(6)	3.09	0.06	3.22	0.11	3.02	0.07	3.09	0.09
(7)	1.97	0.05	2.03	0.04	1.90	0.05	1.93	0.06
(8)	1.09	0.03	1.12	0.04	1.07	0.03	1.09	0.04
(9)	1.97	0.04	1.95	0.07	1.80	0.07	1.90	0.05
(10)	1.93	0.03	1.90	0.07	1.76	0.06	1.87	0.05
(11)	2.52	0.07	2.51	0.06	2.42	0.09	2.44	0.10
(12)	1.18	0.06	1.20	0.10	1.03	0.07	1.10	0.04
(13)	2.16	0.05	2.15	0.10	2.24	0.29	2.11	0.12
(14)	0.24	0.05	0.28	0.07	0.39	0.07	0.27	0.04
(15)	2.34	0.06	2.38	0.06	2.25	0.07	2.31	0.08
(16)	2.90	0.07	2.90	0.08	3.14	0.45	2.86	0.18
(17)	8.60	0.11	8.91	0.13	8.21	0.23	8.50	0.16
(18)	3.04	0.04	3.17	0.05	2.88	0.08	3.00	0.07
(21)	30.70	0.68	31.73	1.24	31.94	0.80	31.83	2.25
(22)	110.04	2.16	110.23	2.25	106.61	2.14	110.04	2.84
(23)	95.02	1.18	94.96	1.63	95.65	1.67	94.87	1.62
(24)	19.48	1.01	20.59	0.72	19.57	0.64	19.91	0.86
(25)	88.32	3.26	88.45	1.68	88.74	1.96	87.79	2.08
(26)	45.84	1.87	46.82	2.05	47.62	1.76	46.62	1.45
(27)	103.19	2.40	102.19	1.63	101.98	1.96	102.60	2.12
(28)	80.33	1.79	78.55	1.37	77.96	2.35	78.22	1.87
(29)	14.17	0.88	14.83	2.98	14.55	0.94	15.02	2.84
(30)	82.01	2.71	82.00	1.94	78.98	3.79	81.78	2.35
(31)	33.79	1.34	33.73	2.45	33.76	2.85	33.46	2.03
hamuli	18.81	0.68	18.21	0.60	18.31	0.80	18.57	0.89

the mean coefficients of variation for the four clusters is less than 5%. While statistically, one could further analyse each morphocluster separately to obtain an index of "local labeling", the biological meaningfulness of such a procedure has little merit in as much as all four of the original morphoclusters were indistinct. The four morphoclusters are biogeographically distributed in the vegetation classification zones of Hou and Hou (1983) as follows: Group 1 occurs in both the temperate deciduous needle-leaved and broad-leaved forests of northeastern China; Group 2 in the subtropical evergreen broad-leaved forests of the southern interior; Group 3 in the temperate

steppe of the southwest; and, Group 4 in the tropical rain forests of the southeast. The results obtained from this expanded dataset confirm those of Tan et al. (2006).

Finally, a hierarchical analysis of the total China database and that of adjacent countries yielded two main clusters (Fig. 5). This reflects the principle that cluster discrimination is greatly affected by geographic scale (Radloff and Hepburn, 2002), and indeed only two morphoclusters emerge from a macroscale analysis of all *A. cerana* (Radloff et al., in preparation). In the linkage distance dendrogram (Fig. 5), it is of interest to note that the Group 3 bees of the Yunnan Province

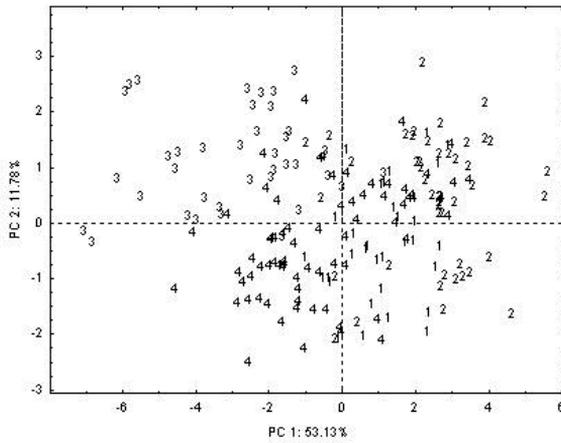


Figure 4. Principal components plot of the morphocluster of *A. cerana* in China using 12 common morphometric characters (Number of colonies $n = 188$). Numbers indicate colonies from regional clusters: 1 = colonies from Jinlin, Liaoning, Beijing and northern Shanxi provinces; 2 = colonies from southern Ganshu and northern and central Sichuan (larger *Aba* bees); 3 = colonies from southern Yunnan, Guangdong, Guangxi, Hong Kong and Hainan and 4 = colonies from the rest of China.

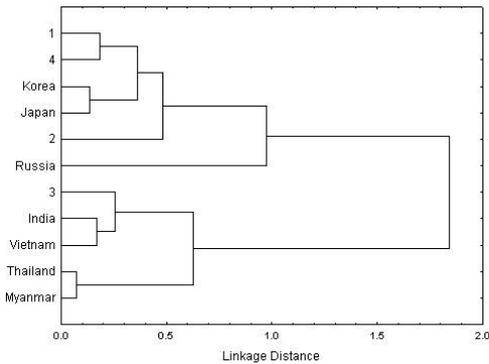


Figure 5. Hierarchical clustering dendrogram for *A. cerana* in China and adjacent countries, derived from single linkage clustering on 12 morphometric characters (Ruttner numbers, (5), (6), (7), (9), (10), (11), (12), (15), (17), B4 (22), D7 (23) and G18 (25)) averaged for regional groups and countries.

show a greater similarity to those of the other sub-Himalayan neighbouring countries than to those of more northerly areas of China, as previously noted by Tan et al. (2003a, b, 2006, 2007).

The morphometric results obtained in the present study are consistent with others on the

correlation between *A. cerana* morphocustering and ecological distribution representative of the major vegetation zones in China (Tan et al., 2006). Our results differ from those of Yang (2001) and Peng et al. (1989); but, given major differences in statistical treatment of data and lack of original raw data, no further comment can usefully be made on this point. The problem of naming these bees is fraught with the long-standing confusion in the literature (about 20 trinomial scientific names have been published for Chinese honeybees alone) and the rigidity of the Linnean system imposed by the International Code of Zoological Nomenclature.

ACKNOWLEDGEMENTS

This project was supported by grants from the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Science, National Natural Scientific Research Program of China-2006 (3067159) to Tan Ken.

Analyse morphométrique multivariée de l'abeille *Apis cerana* de Chine.

Apis cerana / analyse morphométrique / Chine / structure population

Zusammenfassung – Multivariate morphometrische Analyse chinesischer Bienen (*Apis cerana*). Kürzlich wurden von Tan et al. (2001 et seq.) zwei größere Morphokluster von *A. cerana* in China beschrieben, die beide mit bestimmten ökologischen Zonen zusammenfielen. Diese Untersuchungen stützten sich auf eine Sammeldichte von einem Sammelort per 250 000 km² von Nichtwüstenland. Wir haben diese Sammeldichte auf nahezu das Fünffache gesteigert (Abb. 1). Plots der Hauptkomponentenwerte der morphologischen Charaktere der Proben entlang den ersten und zweiten sowie der ersten und dritten Hauptachsen zeigten einen zusammenhängenden Morphokluster an, aus dem zunächst keine weitere Unterstruktur abgeleitet werden konnte. Erst nach einer zusätzlichen lokalen Kennzeichnung wurde deutlich, dass die Bienen der unterschiedlichen Gebiete überlappende regionale Kluster bilden. Dies waren (1) die Bienen von Jinlin, Liaoning, Beijing und den nördlichen Shanxi Provinzen; (2) die größeren Bienen des südlichen Gansu und des nördlichen und zentralen Sichuan; (3) die kleineren Bienen des südlichen Yunnan, Guangdong, Guangxi, Hong Kong und Hainan sowie (4) die Bienen des übrigen China (Abb. 2). Die Eingabe von nur 12 Charakteren in eine schrittweise Diskriminanzanalyse war ausreichend, um die vier Gruppen zu identifizieren (Abb. 3 und 4).

Die *A. cerana* Populationen sind zusammenhängend und panmiktisch. Die morphologische Strukturierung ist undeutlich und der Durchschnitt der mittleren Varianzkoeffizienten in den 4 Gruppen beträgt weniger als 5 %. Plots der ersten und zweiten kanonischen Werte zeigten, dass die Gruppen 1 und 3 sowie die Gruppen 2 und 3 unverbunden sind (Abb. 3, 95 % Konfidenzellipsen). Die quadrierten Mahalanobisabstände zwischen den Zentroiden der Gruppen sind in Tabelle II aufgeführt. Die Mittelwerte der Völker und die Standardabweichungen der 28 für die regionalen Gruppen gemittelten morphometrischen Charaktere ((1), (2), (5) bis (18), Flügeladerungswinkel (21) bis (31) und Hamuli) sind in Tabelle III angegeben.

Eine hierarchische Clusteranalyse anhand der Mittelwerte der gleichen gemeinsamen Charaktere für die regionalen Gruppen 1 bis 4 und der angrenzenden Länder erbrachte ein Dendrogramm mit zwei Hauptklustern. Hierin waren die Völker von Japan und Korea phenetisch zunächst mit denen der chinesischen Regionalgruppen 1 und 4 verbunden, gefolgt von der Regionalgruppe 2 und letztendlich den Bienen von Russland. Die Bienen von Nordvietnam und Nordostindien wurden zuerst mit der chinesischen Regionalgruppe 3 verbunden, gefolgt von den Bienen von Thailand und Myanmar (Abb. 5).

Apis cerana / morphometrische Analyse / China

REFERENCES

- Gong Y.F. (1978) The Chinese honeybee, *Apic. China* 1, 10–13 [in Chinese].
- Gong Y.F. (1983a) Natural beekeeping conditions and honeybee races in China, *J. Fujian Agric. College* 12, 241–249 [in Chinese].
- Gong Y.F. (1983b) Honeybee taxonomy and evolution of honeybee, Fujian Science and Technology Press [in Chinese].
- Hou H.-Y., Hou X.-Y. (1983) Vegetation of China with reference to its geographical distribution, *Ann. Mo. Bot. Gard.* 70, 509–549.
- Johnson R.A., Wichern D.W. (2002) Applied Multivariate Statistical Analysis, Fifth Edition, Prentice Hall, Upper Saddle River, New Jersey.
- Kellogg C.R. (1930) The use of biometry to distinguish between *Apis indica* and *Apis mellifica*, *Bee World* 11, 127–130.
- Kellogg C.R. (1936) Biometric studies of the Chinese honey bee (*A. indica*), *Arch. Bienenkd.* 17, 36–38 [in German].
- Kellogg C.R. (1938) Biometrical studies on the wings of the Chinese honey bee, *Fukien Christian Univ. Sci. J.* 1, 1–8.
- Kellogg C.R. (1941) Some characteristics of oriental honeybee (*Apis indica* F.) in China, *J. Econ. Entomol.* 34, 717–719.
- Kellogg C.R. (1968) Entomological excerpts from southeastern China, Claremont Manor, California.
- Kellogg C.R., Yung L.L. (1929) Studies in the variation in number of hooks on the hind wings of the Chinese honey bee (*Apis indica*), *Proc. Nat. Hist. Soc. Fukien Christian Univ.* 2, 57–59.
- Kuang B.Y. (1986) Varieties of *Apis* in China, *Apic. China* 5, 7–9 [in Chinese].
- Kuang B.Y. (1988) Comparative studies on the biology of six honeybee species, *Apic. Sci. Tech.* 1, 12–14 [in Chinese].
- Kuang B.Y., Li Y.Q. (1988) Studies on Apini, *Sci. Agric. Sinica* 21, 85–90 [in Chinese].
- Liu Y., Liang R., Lai Y.S. (1982) A report on the Chinese honeybee in Guangdong Province, Institute of Honeybee Science Research Kunming [in Chinese].
- Meixner M. (1994) Analyse polymorpher Subspecies von *A. mellifera* L.: Morphometrische und molekulare Untersuchungen an den europäischen Rassen *A. mellifera carnica* und *ligustica* und den afrikanischen Rassen *A. mellifera monticola* und *scutellata*, PhD thesis, Fachbereich Biologie der J. W. Goethe-Universität, Frankfurt am Main, Germany.
- Peng Y.S., Nasr M.E., Locke S.J. (1989) Geographical races of *Apis cerana* Fabricius in China and their distribution. Review of recent Chinese

- publications and a preliminary statistical analysis, *Apidologie* 20, 1–9.
- Quinn G.P., Keough M.J. (2002) *Experimental Design and Data Analysis for Biologists*, Cambridge University Press, Cambridge.
- Radloff S.E., Hepburn H.R. (1998) The matter of sampling distance and confidence levels in the subspecific classification of honeybees, *Apis mellifera* L., *Apidologie* 29, 491–501.
- Radloff S.E., Hepburn H.R. (2002) Multivariate morphometric analysis of the *Apis cerana* populations of southern Asia, Proc. 14th Int. Congr. IUSSI, Sapporo, p. 209.
- Rao P.V. (1998) *Statistical Research Methods in Life Sciences*, Brooks/Cole Publishing Company, Pacific Grove, CA.
- Ruttner F. (1988) *Biogeography and taxonomy of honeybees*, Springer, Berlin.
- Ruttner F., Tassencourt L., Louveaux J. (1978) Biometrical-statistical analysis of the geographic variability of *Apis mellifera* L. 1. Materials and methods, *Apidologie* 9, 363–381.
- StatSoft, Inc. (2006) STATISTICA, version 8.1, www.statsoft.com.
- Tan K., Zhang X. (2001) Morphological study of *Apis cerana* in Yunnan, *J. Bee* 27, 2–4 [in Chinese].
- Tan K., He S.Y., Zhang X. (2002a) MtDNA variation of the eastern honeybee *Apis cerana* in Yunnan, *Apic. China* 53, 4–6 [in Chinese].
- Tan K., He S.Y., Zhang X. (2002b) Study on the correlation between morphometric characters and environmental factors of *Apis cerana* in Yunnan, *J. Bee* 28, 5–7 [in Chinese].
- Tan K., Zhang X., He S.Y., Zhou D.Y. (2003a) Morphometrics and biogeography of *Apis cerana* in Yunnan, *Apic. China* 53, 4–6 [in Chinese].
- Tan K., Fuchs S., Koeniger N., Ruiguang Z. (2003b) Morphological characterization of *Apis cerana* (Fabr.) in the province Yunnan of China, *Apidologie* 34, 553–562.
- Tan K., Zhang X., He S.Y., Zhou D.Y. (2005) Morphometric study of *Apis cerana* in China, *J. Yunnan Agric. Univ.* 53, 4–6 [in Chinese].
- Tan K., Mexiner D.M., Fuchs S. (2006) Morphological and molecular studies of *Apis cerana* Fabr. (Apidae, Hymenoptera) in China, *Syst. Biodiv.* 4, 472–482.
- Tan K., Natapot W.N., Smith D.R. (2007) Mitochondrial DNA diversity of China *Apis cerana*, *Apidologie* 38, 238–246.
- Xun Q. (1984) An investigation of the Chinese honeybee in the southern mountainous areas of Tibet, *Apic. China* 4, 19 [in Chinese].
- Yang G.H. (1982) Survey of the Chinese honeybee in Ganzi area in Sichuan, in: *Selected References on the Chinese Honey Bee*. Gansu Provincial Apicultural Research Institute, Tianshui [in Chinese].
- Yang G.H. (2001) *The biology of Apis cerana*, G.H. Yang, Beijing [in Chinese].
- Yang G.H., Xu S.Y. (1982) The distribution and geographical variation of *Apis cerana sinensis* in China (Part 1), *Zhongguo Yangfeng* 3, 17–19 [in Chinese].
- Yang G.H., Li G.H. (1983) The distribution and variations of the Chinese honey bee. V. The survey of the Chinese honeybee in the northeastern regions, *Zhongguo Yangfeng* 5, 19 [in Chinese].
- Yang G.H., Zhang Q.H. (1995) Distribution, variation and characteristics of *Apis cerana cerana* in the northwest altiplano of Sichuan Province, *Sci. Agric. Sinica* 28 (A01), 202–206 [in Chinese].
- Zhen-Ming J., Guanhang Y., Shuangxiu H., Shikui L., Zaijin R. (1992) The advancement of apicultural science and technology in China, in: Verma L.R. (Ed.), *Honeybees in mountain agriculture*, Oxford & IBH Publishing Co Pty Ltd, New Delhi, pp. 133–147.
- Zhuang D.A. (1982) Studies on the morphological characteristics of the Chinese honey bee in various areas of Guizhou province, in: *Selected References on the Chinese Honey Bee*, Gansu Provincial Apicultural Research Institute, Tianshui: Gansu, pp. 231–227 [in Chinese].