Apidologie 32 (2001) 435–447

Original article

Morphometric analysis of *Apis cerana* populations in the southern Himalayan region

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(Received 6 February 2001; revised 23 May 2001; accepted 29 June 2001)

Abstract – Multivariate analyses of morphometric traits of *Apis cerana* were measured from 3704 individual workers of 279 colonies from 64 localities with an average sampling distance resolution of 50 km along a 2200 km transect in the southern Himalayan region bordered by Pakistan to the west and Myanmar to the east. Factor and discriminant function analyses revealed four major morphoclusters (= unnamed subspecies), two of which are further subdivisable into three biometric subgroups each. These morphoclusters can only be partially integrated into any current subspecific nomenclature available for *Apis cerana*. Morphocluster separation is related to physiographic differences which create a partial temporal reproductive isolation associated with altitude. High variance domains occur at the edges of morphocluster and biometric groupings. The bees decrease in size from west to east but increase in size with increasing altitude.

***Apis cerana* / honeybees / morphoclusters / population genetics / southern Himalayas**

1. INTRODUCTION

In many ways *A. cerana* Fabr. is the oriental equivalent of its occidental counterpart *A. mellifera* L., both having equally wide areas of distribution and spectra of adaptations (Ruttner, 1988). However, multivariate morphometric analyses of the former lag behind the latter and a recent survey of the literature shows that infraspecific classification and population structure in *Apis cerana* are fraught with difficulties (Hepburn et al., 2001). A beginning toward a resolution of this disparity is apparent from the recent publication of several such analyses for different parts of Asia (Sasaki, 1994;

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Deowanish et al., 1996; Damus and Otis, 1997; Sylvester et al., 1998; Sihanuntavong et al., 1999; de la Rúa et al., 2000; Smith et al., 2000; Tilde et al., 2000).

The southern Himalayan region and surrounds are particularly interesting in this context because analyses of morphological variation extend well into the past (e.g. Kapil, 1956; Narayanan, 1960, 1961a,b; Kshirsagar, 1973). While the earlier studies were univariate in approach, some more recent works have been fully multivariate in the analysis of Himalayan honeybees (Verma et al., 1989, 1994; Singh et al., 1990; Singh and Verma, 1993). Although this suite of four papers treated the western, central and eastern subregions, some rather large gaps precluded a unified multivariate analysis of the whole, contiguous region. The recent acquisition of substantially more data from former lacunae now permits a complete multivariate morphometric analysis of population structure in *A. cerana* in the southern Himalayan region, extending from the northeastern border of Pakistan to the northwestern border of Myanmar at an average interlocality resolution of 50 km. This allows for extensive analyses of the honeybee size-altitude relationship, delineation of clines, correlations between morphocluster distribution and physiography, and the nature of morphometric high variance domains.

2. MATERIALS AND METHODS

2.1. Honeybees

The workers of *A. cerana* used in this analysis include all the original unpublished raw data for 44 localities used in previous studies of the southern Himalayan region (Verma et al., 1989, 1994; Singh et al., 1990; Singh and Verma, 1993) (Tab. I). In these studies sample sizes were 60 bees/colony and 4–5 colonies/locality for the western and central subregions and 10 to 20 bees/colony and 3–6 colonies/locality in the eastern sector. In addition honeybees from 20 new localities were collected and 20 bees/colony and 3–4 colonies/locality were measured (Tab. I). All these localities are also mapped (Figs. 1 and 2).

Table I. Localities from which *A. cerana* were collected along a 2 200 km transect in the southern Himalayan region (* new localities).

No.	Localities	Coordinates		Altitude	Swarming time
1.	Kupwara	34.31N	74.16E	1811	April
2.	Gurais	34.37N	74.53E	2364	April
3.	Tral	33.56N	75.10E	2007	April
4.	Dras	34.26N	75.46E	2977	April
5.	Srinagar	34.08N	74.50E	1768	April
6.	Sonamarg	34.18N	75.21E	2740	April
7.	Rajouri	33.23N	74.21E	938	February
8.	Kishtwar	33.19N	75.48E	1664	April
9.	Dalhousie	32.32N	75.58E	2036	April
10.	Kangra	32.05N	76.16E	700	February
11.	Katrain	32.05N	77.08E	1463	March
12.	Pooh	31.40N	78.34E	2837	April
13.	Bhareri	31.23N	76.34E	1007	March
14.	Mandi	31.43N	76.50E	761	February
15.	Roghi	31.32N	78.15E	3017	April
16.	Bilaspur	31.15N	76.40E	587	February

Table I. (Continued).

No.	Localities	Coordinates		Altitude	Swarming time
17.	Bagi	31.15N	77.27E	2648	April
18.	Shimla	31.07N	77.10E	2206	April
19.	Solan	30.50N	77.08E	1530	April
20.	Nahan	30.33N	77.21E	905	February
21.	Dehradun*	30.30N	78.08E	762	February
22.	Haridwar*	30.02N	78.04E	330	February
23.	Pauri*	30.12N	78.48E	1550	April
24.	Chaubattia*	29.55N	79.00E	2122	April
25.	Almora*	29.36N	79.40E	≈1750	April
26.	Nainital*	29.23N	79.32E	1920	April
27.	Jeolikote*	29.16N	79.46E	1250	March
28.	Haldwani*	29.13N	79.31E	≈800	February
29.	Budaun*	28.02N	79.07E	≈150	February
30.	Lucknow*	26.50N	80.54E	≈150	February
31.	Lohaghat*	29.25N	80.06E	≈1200	March
32.	Lali*	29.49N	80.36E	1097	March
33.	Sharmali*	29.14N	80.25E	1635	April
34.	Navadurga*	29.15N	80.27E	1837	April
35.	Lanakedareshwar*	29.22N	80.56E	1360	March
36.	Durg*	29.10N	81.20E	1345	March
37.	Ghughuti*	29.18N	82.12E	2470	April
38.	Vinaula*	29.18N	82.18E	2895	April
39.	Liwang	28.25N	82.40E	1500	April
40.	Laltibang	28.20N	82.30E	900	February
41.	Ghorahi	28.05N	82.20E	400	February
42.	Lumle	28.20N	84.00E	1400	March
43.	Tansen	27.55N	83.40E	1067	March
44.	Sidheshwar*	27.18N	85.56E	1463	March
45.	Suspa*	27.44N	86.10E	1600	April
46.	Letang	26.40N	87.25E	250	February
47.	Homtang	27.10N	87.10E	600	February
48.	Virgaon	27.05N	87.51E	1200	March
49.	Kurseong	26.56N	88.20E	1458	March
50.	Gangtok	27.02N	88.40E	1818	April
51.	Bongaigaon	26.28N	90.32E	76	February
52.	Khoirabari	26.38N	91.51E	134	February
53.	Guwahati	26.11N	91.47E	56	February
54.	Shillong	25.34N	91.56E	1496	March
55.	Silchar	24.50N	92.51E	67	February
56.	Aizawl	23.45N	92.45E	1132	March
57.	Itanagar	27.08N	93.40E	550	February
58.	Dimapur	25.54N	93.48E	160	February
59.	Mao	25.30N	94.07E	2012	April
60.	Kohima	25.41N	94.06E	1495	March
61.	Ukhrul	25.08N	94.23E	1829	April
62.	Imphal	24.49N	93.58E	792	February
63.	Moirang	24.30N	93.48E	782	February
64.	Churachandpur	24.20N	93.40E	914	February

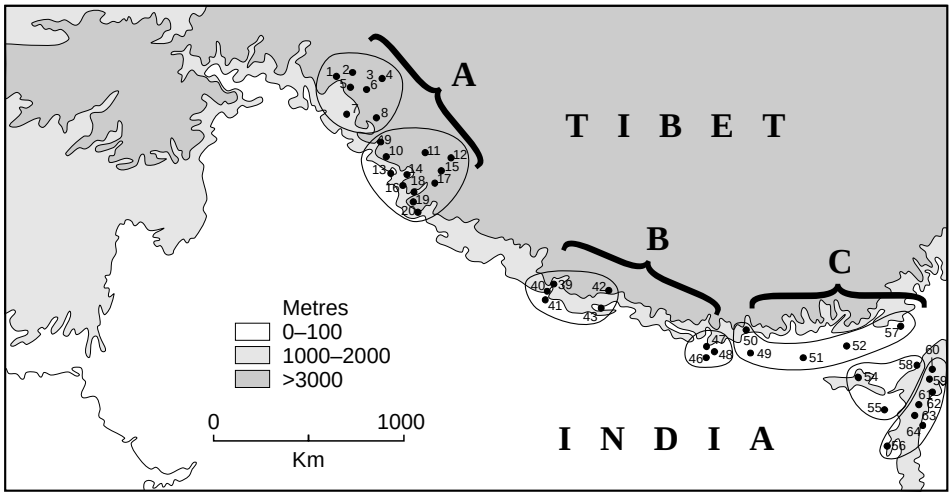

Figure 1. Composite distribution of the biometric groups of *A. cerana* previously identified in the southwestern (Verma et al., 1989, 1994) and southeastern (Singh et al., 1990; Singh and Verma, 1993) Himalayan subregions. Locality numbers are the same as in Figure 2 and details given in Table I. The biometric groups from west to east are: A-(1) Kashmir, (2) Himachal Pradesh, B-(3) Nepali Terai plains, (4) Nepali Midland Hills, C-(5) Himalaya (Sikkim, west Bengal, Assam and Arunachal Pradesh), (6) Brahmaputra (Assam and Meghalaya) and (7) Nagaland (Nagaland, Manipur and Mizoram).

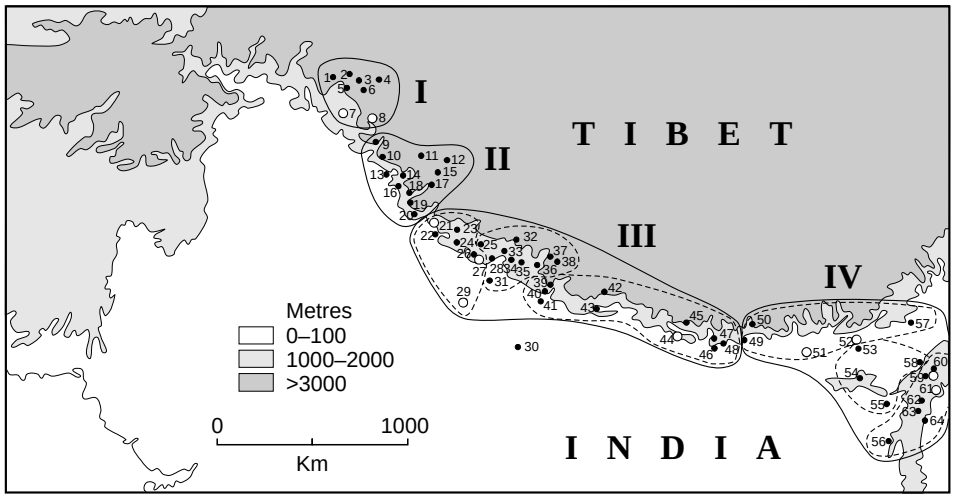

Figure 2. Morphoclusters of *A. cerana* obtained by multivariate analysis of bees of the southern Himalayan region along a 2 200 km transect with a 50 km interlocality sampling resolution between 64 localities. The morphoclusters occur as follows: I Kashmir; II Himachal Pradesh; III Uttar Pradesh and Nepal; IV Sikkim, Bengal and NE states of India. Morphoclusters III and IV are further resolved into three biometric subgroups each from west to east as follows: IIIa Uttar Pradesh, IIIb W. Nepal, IIIc central and E. Nepal; IVa Sikkim, Bengal, Assam and Arunachal Pradesh, IVb Assam and Meghalaya, IVc Nagaland, Manipur and Mizoram. Names of localities are given in Table I. Open circles denote localities of significantly high morphometric variance.

2.2. Measurements and analysis

Fifty-five quantitative morphological characters previously used (Verma et al., 1989, 1994; Singh et al., 1990; Singh and Verma, 1993) were measured for the new localities in this study. The first statistical procedure was to perform a factor analysis on the colony means using these 55 characters for all 3704 bees collected across the entire transect. This procedure established which characters have larger loadings in the various factors and allows the parsimonious reduction in the number of characters actually needed for further analysis. The next procedure was a correlation analysis of the colony means for all 55 characters to determine which characters were highly correlated. If two or more characters with high factor loadings (greater than 0.6) were highly correlated ($r > 0.8$), then only one was selected for further analyses. Finally, a factor analysis for the whole transect using colony means for the remaining 22 selected characters was performed, followed by a discriminant analysis of colony means for these 22 characters to determine colony groupings (morphoclusters). A jackknife procedure was used to classify each colony into a morphocluster with the highest posterior probability according to the discrimination function (Lachenbruch and Mickey, 1968). Wilks' Lambda statistic was used to test for significant differences between the vector of means of the characters entered into the discriminant functions. The intercolonial variances at each locality were tested for heterogeneity by means of Levene's F Statistic (Johnson and Wichern, 1998).

3. RESULTS

The factor and correlation analyses procedures using the colony means of the 55 morphometric characters of worker honeybees from the 64 localities resulted in the following 22 morphometric characters for further analysis: (numbers in brackets refer to schemes of Verma et al., 1989, 1994; Singh et al., 1990; Singh and Verma, 1993): forewing-length of radial cell (1), length of apical position of radial cell (4), length of forewing (5); angles of venation- (10), (11), (13); hindwing-length of basal portion of radial vein (20), number of hamuli (26), length of vannal lobe (29); hindleg-length of femur (30), length of tibia (31), length of metatarsis (32); tongue-total length of tongue (36), length of labial palp (37); abdomen-total length of 3rd tergite (42), length of dark band of 4th tergite (44), total length of 4th tergite (45), length of wax mirror on 3rd sternite (48), total length of 3rd sternite (49), length or depth of 6th sternite (50); antenna-length of flagellum (54), total length of antenna (55).

A factor analysis using the colony means of the selected 22 characters of worker honeybees from the entire transect isolated three factors with eigenvalues greater than one. Factor 1: characters associated with the size of the honeybees; Factor 2: angle of venation (10); and Factor 3: angles of venation (11) and (13). These three factors accounted for 78.6% of the variance in the data. The loadings for each character had an absolute value greater than 0.6. A graph of the factor scores revealed four morphoclusters, those colonies from the Kashmir region forming one cluster (I), those colonies from the Himachal region forming a second cluster (II), those from Uttar Pradesh and Nepal regions forming a third cluster (III), and those from the northeast Himalayan region forming a fourth cluster (IV) (Figs. 2 and 3). The colonies from two localities at high altitudes, Ghughuti (2470 m) and Vinaula (2895 m), formed their own cluster (Fig. 3). The 5 colonies from Lucknow (30) appeared in the far left-hand of the third morphocluster indicating that the bees from this region are smaller and probably belong to a southern Indian morphocluster not defined here (Fig. 3). The graph of the factor scores confirms the results of Verma et al. (1989, 1994) that Nepali bees are smaller in size than

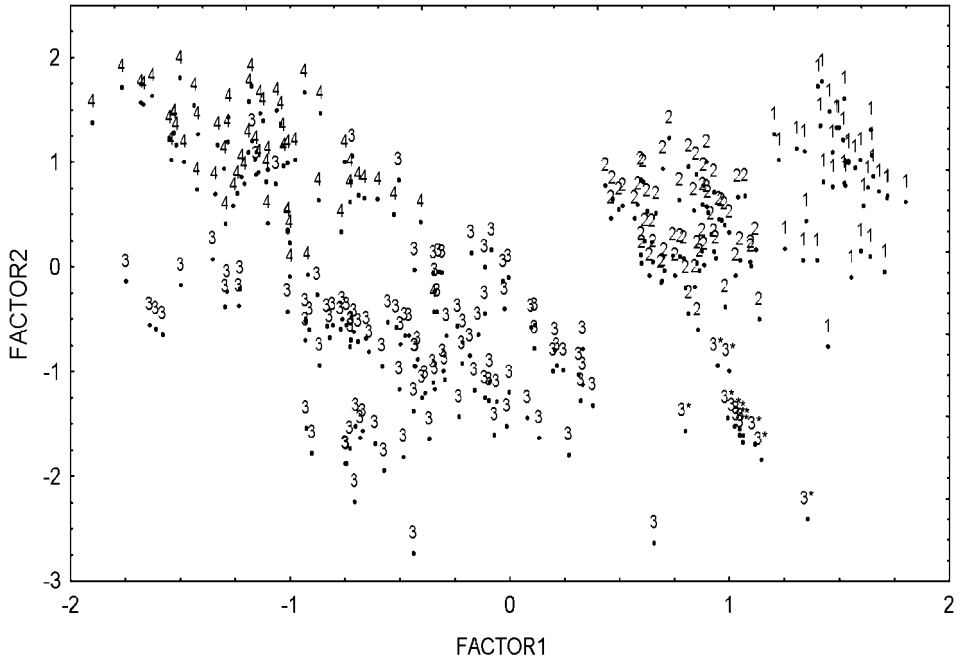

Figure 3. Factor analysis plot of the southern Himalayan morphoclusters of *A. cerana* using 22 morphometric characters: morphocluster I comprises colonies from Kashmir; morphocluster II comprises colonies from Himachal Pradesh; III of colonies from Uttar Pradesh and Nepal and IV colonies from the northeast Himalayan region. Morphoclusters in figure are represented in Arabic numbers to avoid confusion of overlapping amongst Roman numbers. The colonies from Ghughuti and Vinaula are denoted by 3*.

Kashmiri and Himachali bees, but bigger than Manipuri bees.

A discriminant analysis confirmed the four morphoclusters with 100% correct classification of the 39 colonies from the Kashmir region with a posteriori probabilities equal to one for all colonies. Likewise, there was a 100% correct classification of the 60 colonies from the Himachal region with a posteriori probabilities equal to one for all colonies. Also, there was a 97.5% correct classification of the 121 colonies from Uttar Pradesh and Nepal regions (3 misclassified from Almora into morphocluster IV) with a posteriori probabilities equal to 1 for 111 colonies, $0.990 < P < 0.996$ for 3 colonies, $0.95 < P < 0.98$ for 2 colonies, $P = 0.82$ for 1 colony and $P = 0.51$ for 1 colony. Finally there was a 98.3% correct classification of the 59 colonies from the northeast Himalayan region (1 misclassified from Bongaigaon into morphocluster III) with a posteriori probabilities equal to one for all colonies. The overall classification rate was 98.6%. The jackknife procedure gave the same classification results, except one more colony from Almora was classified incorrectly into morphocluster IV; i.e., 97.8% correct classification rate. A significant difference was found among the means of the four clusters [$\Lambda = 0.0002$ with (17, 3, 275) df; $F = 249.7$ with (51, 771) df, $P < 0.0001$]. Seventeen of the 22 morphometric characters which entered into the discriminant function in descending order of discriminating power are as follows: (48), (37), (1), (36), (10), (29), (45), (11), (13),

(5), (55), (54), (31), (30), (44), (50), and (42). The means and standard deviations of the 22 characters for each morphocluster are given in Table II.

A factor analysis carried out using the 59 colonies from the northeast Himalayan region alone (morphocluster IV) at a mean sampling distance resolution of approximately 50 km showed morphological differences amongst the localities. Three biometric groups were found, group (a) comprising colonies from the foothills of

Table II. Means and standard deviations (sd) of 22 morphometric characters (measurements in mm, angles in degrees) for four morphoclusters of southern Himalayan *A. cerana*.

	Morphoclusters							
	I *n* = 39		II *n* = 60		III *n* = 121		IV *n* = 59	
Character	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Forewing								
(1)	3.18	0.03	3.01	0.03	2.97	0.09	2.89	0.06
(4)	1.95	0.04	1.85	0.03	1.81	0.05	1.76	0.04
(5)	8.75	0.05	8.50	0.08	8.28	0.29	8.21	0.15
Angle								
(10)	107.67	1.59	107.50	1.42	104.80	2.33	109.57	2.33
(11)	95.66	1.66	93.78	1.17	93.11	2.17	93.85	1.66
(13)	90.55	1.11	88.37	0.83	91.09	4.93	88.15	1.73
Hindwing								
(20)	1.48	0.02	1.44	0.02	1.36	0.05	1.33	0.03
(26)	19.44	1.02	19.36	0.91	17.72	0.66	18.20	0.79
(29)	1.24	0.03	1.20	0.03	1.12	0.03	1.14	0.02
Hindleg								
(30)	2.58	0.03	2.47	0.03	2.34	0.12	2.30	0.03
(31)	3.20	0.03	3.09	0.03	2.90	0.03	2.88	0.02
(32)	2.01	0.03	1.94	0.03	1.81	0.08	1.79	0.03
Tongue								
(36)	5.35	0.14	5.31	0.13	4.46	0.48	4.65	0.08
(37)	2.75	0.07	2.55	0.04	2.12	0.12	2.13	0.04
Abdomen								
(42)	2.10	0.02	1.98	0.03	1.75	0.20	1.70	0.04
(44)	1.20	0.03	1.09	0.03	0.95	0.10	0.88	0.10
(45)	2.06	0.03	1.90	0.02	1.73	0.07	1.67	0.05
(48)	1.24	0.02	1.52	0.01	1.10	0.06	0.93	0.04
(49)	2.60	0.02	2.43	0.03	2.29	0.08	2.25	0.06
(50)	2.40	0.02	2.32	0.04	2.16	0.07	2.13	0.05
Antenna								
(54)	2.72	0.02	2.54	0.02	2.31	0.16	2.49	0.03
(55)	4.31	0.02	4.11	0.03	3.70	0.15	3.78	0.04

the Himalayas, group (b) comprising colonies from the Brahmaputra valley and Khasi Hills, and group (c) colonies from the Naga and Mizo Hills (Fig. 2). The factor analysis isolated six factors with eigenvalues greater than one. The first three factors associated with size of the bees; factors 4 and 5 associated with angles of wing venation; and factor 6 having the number of hamuli (26) as the larger factor loading character.

The factor scores plot showed that the bees from the Naga and Mizo Hills are smaller than those of the Brahmaputra Valley, the Khasi Hills and from the foothills of the Himalayas. A discriminant analysis confirmed the three biometric groups with 100% correct classification of the colonies into each group. The jackknife procedure gave the same classification results i.e. 100% overall correct classification rate. A significant difference was found between the means of the three biometric groups ($\Lambda = 0.02658$ with (8, 2, 56) df, $F = 31.5$ with (16, 98) df, $P < 0.0001$). These results support the findings of Singh et al. (1990). Eight of the 22 morphometric characters which entered the discriminant function in descending order of discriminatory power are (48), (50), (49), (4), (31), (20), (42), and (10).

A factor analysis carried out using the 121 colonies from the Uttar Pradesh and Nepal regions alone (morphocluster III) at a mean sampling distance resolution of approximately 50 km also revealed morphological differences amongst the localities. Six factors with eigenvalues greater than one were isolated accounting for 77.4% of variation in the data. The factor scores plot delineated three biometric groups: the colonies from northwestern Uttar Pradesh (localities 21–27, 29, excluding 25) forming group (a); those from northeastern Uttar Pradesh and western Nepal (localities 25, 28, 31–36) forming a second group (b); and those from central and eastern Nepal (localities 39–48) group (c) (Fig. 2). The colonies from Ghughuti 37 and Vinaula 38 were assigned to the right-hand half of the factor plot indicating that larger bees were present at these two higher altitude localities in Nepal.

A discriminant analysis supported the three biometric groups with 100% correct classification of the 19 colonies into group (a) (northwestern Uttar Pradesh), 100% correct classification of the 51 colonies into group (b) and 95.8% correct classification of the 48 colonies (2 colonies misclassified) into group (c). The overall classification rate was 98.3% which reduced to 95.9% when the jackknife procedure was applied. Ten morphometric characters entered the discriminant function in descending order of discriminatory power (49), (48), (13), (20), (54), (36), (11), (55), (5), and (4). A significant difference was found between the means of the three biometric groups ($\Lambda = 0.0257$ with (10, 2, 118) df, $F = 57.1$ with (20, 218) df, $P < 0.0001$).

Finally, a factor analysis and discriminant analysis carried out on the colonies from the Kashmir region, Himachal region, Nepal (localities 39–48), and Manipur with a mean sampling distance resolution between regions of approximately 480 km revealed four distinct morphoclusters. Three factors with eigenvalues greater than one were isolated, which accounted for 86.2% of the variation in the data, and 12 morphometric characters entered the discriminant function in descending order of discriminatory power (48), (45), (54), (13), (1), (55), (37), (10), (20), (36), (49), and (26). 100% correct classification of the colonies into all 4 morphoclusters was established and verified by the jackknife procedure.

The intercolonial variances at the 64 localities were determined using the first factor loadings. Significantly higher variations were found at the following localities: 7 and 8 (between morphoclusters I and II); 21 (between morphoclusters II and III); 27 and 29 (between biometric groups 1 and 2 within morphocluster III); 44 (in biometric group 3 within morphocluster IV); and 60

and 61 (in biometric group 2 within morphocluster IV) (Levene's F = 2.16 with (63, 215) df, $P < 0.0001$).

Significant positive correlations were found between the morphological characters associated with the size of the honeybees and the altitudes of the localities at which they were sampled. For example, the correlation between the forewing length (5) and altitude using all 64 localities was highly significant ($r = 0.63$, $P < 0.0001$). The results confirm the findings of Verma et al. (1994) for groups I and II, Verma et al. (1989) for group III and Singh et al. (1990) for group IV.

4. DISCUSSION

Elucidation of the complex structure of *A. cerana* populations in the southern Himalayan region is facilitated in a graphical, historical context (Fig. 1). Earlier studies demonstrated that honeybees of the western subregion were generally larger and darker with increasing altitude (Kapil, 1956; Narayanan, 1960, 1961a,b; Kshirsagar, 1973). Subsequent univariate analyses suggested two distinct morphoclusters, one in Kashmir the other in Himachal Pradesh (Mattu and Verma, 1983, 1984a,b), a conclusion also reached in later multivariate analyses (Verma et al., 1994). Interestingly, there were no intermediate or clinal forms between these two clusters.

Two additional morphoclusters were described from the central Himalayan region of Nepal, one from the Terai plains, the other from the midland hills (Fig. 1). Again, correlations between some morphological characters and altitude were reported (Verma et al., 1989). Moreover, the two Nepali clusters were shown to be distinctly different from the clusters of Kashmir and Himachal Pradesh to the west and those of the east in Nagaland (Manipuri bees). However the inter-cluster average sampling distance was about 500 km (Fig. 1). A further analysis of the eastern subHimalayan region yielded three additional morphoclusters designated as Nagaland, Brahmaputra, and Himalaya (Singh et al., 1990; Singh and Verma, 1993; Fig. 1). Verma (1995) also noted that the bees became progressively smaller from west to east. Interestingly, this clinal pattern is also mirrored in a north-south cline for the Indian subcontinent which Diniz-Filho et al. (1993) observed in a re-analysis of the Kshirsagar (1981) data set. While the above analyses were multivariate in approach, they were nevertheless statistically independent and unrelated studies of the western, central, and eastern Himalayan subregions with large interregional data gaps.

There are four statistically discrete morphoclusters for the whole region (Fig. 2). Three of these morphoclusters (cluster I Kashmir, II Himachal Pradesh and IV Sikkim, Bhutan, Arunachal Pradesh, Assam, Meghalaya and Nagaland) confirm conclusions reached in earlier, unrelated studies (Verma et al., 1989, 1994; Singh et al., 1990; Singh and Verma, 1993). Likewise, the three biometric subgroups of cluster IV representative of Himalaya, Brahmaputra, and Nagaland confirm the interpretations of others (Singh et al., 1990). The major departures from the morphoclusters obtained in previous studies (compare Figs. 1 and 2) essentially arise from the filling of previous lacunae in sampling Uttar Pradesh and the greater part of Nepal. Now that these gaps have been filled, a more contiguous run of data show that Uttar Pradesh and Nepal together actually form morphocluster III which is statistically quite discrete from its neighbours. Moreover, this morphocluster is further subdivisable into three biometric groups consisting of the bees of (a) W. Uttar Pradesh, (b) E. Uttar Pradesh and W. Nepal, and, (c) central and eastern Nepal (Fig. 2).

Thus the composite structure for *A. cerana* in the Himalayan region reveals four primary, major morphoclusters (= subspecies?), two

of which can be further resolved into three biometric subgroups (Fig. 2). These results are the products of statistical analysis of morphological variation for the region. In reflecting on the clusters shown in Figure 1, Verma (1995) suggested that the morphological groups might well be biologically meaningful because they are geographically separated. However, in biological terms the four morphoclusters and six biometric subgroups of Figure 2 still require explanations as to their biological meaningfulness as separate populations.

The analysis of the climatic zones for this botanically palaeotropical part of Eurasia shows that the whole region has a warm temperate, rainy climate with dry winters in the system of Köppen and Geiger (cf. Müller, 1982); alternatively, in the system of Troll and Paffen (cf. Müller, 1982), Kashmir alone falls into the subtropical steppe while the rest of the transect from Himachal Pradesh through Nagaland is classically tropical but with varying rainfall. Physiographical changes in the southern Himalayan region are quite pronounced with respect to altitude and, indeed, increases in honeybee size are correlated with increasing altitude. This physiographic differentiation is strongly reflected in defining the boundaries of the four major morphoclusters because the seasonality of reproductive swarming varies with altitude, not longitude, in the southern Himalayan region (Tab. I). Consequently, whichever the morphocluster region, swarming usually begins in mid-February in lowland valleys and on plains below 1000 m. At intermediate altitudes (1000–1500 m) swarming commences in mid-March and at higher and more temperate levels (1500–3000 m) swarming is delayed until early April (Verma, unpublished observations).

The effect of this variation for the onset of swarming is that significant temporal reproductive barriers exist between adjacent morphoclusters of *A. cerana*. For example, in the morphocluster I region 88% of swarming begins in April while for morphocluster II 50% of swarming begins in February/March ($\chi^2 = 7.74$, 2 df, $P = 0.0208$). A combination of clusters I and II shows that 65% of swarming begins in April while in region III 61% occurs in February/March. Swarming in the morphocluster III region is also significantly different from that of IV ($\chi^2 = 10.60$, 4 df, $P = 0.0314$). So while there is certainly a degree of temporal overlap in the swarming periods of adjacent morphoclusters of *A. cerana*, there are also substantial and significant periods of temporal isolation between them as well. This effect has also been noted for morphoclusters of *A. mellifera* (Hepburn and Radloff, 1998).

In the analysis of honeybee population structure considerable insight about genetic variability and gene flow is reflected in the variance characteristics of a particular trait. In the case of African *A. mellifera*, variance domains occur at transitions between differing ecological or climatological zones and represent zones of hybridization (Hepburn and Radloff, 1998). In the case of southern Himalayan *A. cerana*, ten high variance domains occur (Fig. 2) nearly all of which are situated at or near borders between morphoclusters (21, 29), their biometric subgroups (27, 51, 52, 60), or areas of rapid physiographic change (7, 8). In the absence of complete temporal reproductive isolation, it is inferred that these high variance areas result from introgressive hybridization between adjacent morphoclusters and biometric groups. Such an interpretation could benefit from additional confirmation using DNA probes.

A final matter concerns subspecific classification of these *A. cerana* honeybees. In a recent review of this problem it was shown that subspecific categories are riddled with anomalies and are biologically tenuous at best (Hepburn et al., 2001). Previously, Engel (1999) analysed and corrected the subspecific nomenclature for *A. cerana* by rigorous application of the rules of the International Code of Zoological Nomenclature. This was a matter of rules for names, not

comment on biological entities. In any event, in his assessment the two morphoclusters representing Kashmir and Himachal Pradesh would be *A. cerana cerana* while both morphoclusters III and IV would be the subspecies *A. cerana skorikovi*. However, all four morphoclusters obtained by multivariate morphometric analysis (Fig. 2, Tab. II) enjoy equal statistical and morphometric status and all four would fully qualify as morphological subspecies of *A. cerana* just as for subspecies of *A. mellifera* (Ruttner, 1988). We believe that it is too soon to assign ICZN-based names to morphoclusters (= subspecies?) of *A. cerana* in general; and, for the Himalayan region, believe that it is first necessary to characterize their honeybee neighbours in Tibet to the north, Myanmar in the east, India to the south and Pakistan and Afghanistan to the west. Moreover, the philosophical and practical basis for the subspecies concept is fraught with problems and should probably be abandoned (e.g., Wilson and Brown, 1953).

ACKNOWLEDGEMENTS

We thank M. Engel, P. Neumann and D.R. Smith for constructive discussions on this manuscript.

Résumé – Analyse morphométrique des populations d'*Apis cerana* du versant sud de l'Himalaya. Des échantillons d'ouvrières d'*Apis cerana* Fabr. ont été prélevés le long d'un transect de 2200 km allant du Pakistan au Myanmar sur le versant sud de l'Himalaya. Leurs caractéristiques morphométriques ont été étudiées à l'aide de l'analyse factorielle et de l'analyse discriminante d'une analyse multivariée. Au total 3704 abeilles provenant de 64 colonies ont été étudiées individuellement. La résolution de la distance d'échantillonnage était de 50 km (Tab. I). Vingt-deux caractères sur les 55 mesurés ont suffit à séparer des « morphoclusters » (groupes caractérisés par un ensemble de caractères morphométriques).

Quatre morphocluters ont été déterminés : I Cachemire, II Himachal Pradesh, III Uttar Pradesh et Népal occidental et IV Népal central et oriental, Sikkim, Arunachal Pradesh, Bengale et Nagaland (Figs. 2 et 3). En outre les morphoclusters II et III ont été subdivisés en trois sous-groupes biométriques : III (a) Uttar Pradesh du nord-ouest, (b) Uttar Pradesh du nord-est et Népal oriental et (c) Népal occidental ; IV (a) avant-monts orientaux de l'Himalaya au Sikkim, Arunachal Pradesh central et Népal oriental, (b) vallée du Brahmapoutre et collines de Khasi, (c) Nagaland, Manipur et Mizoram (Fig. 2).

Ces résultats proviennent d'une combinaison de données précédemment publiées pour 44 localités (Verma et al., 1989, 1994 ; Singh et al., 1990) et de données provenant de 20 localités complémentaires qui ont comblé des lacunes cruciales pour le transect du versant sud de l'Himalaya. D'après leur statut statistique les quatre morphoclusters définis ici correspondent à des sous-espèces séparées (mais non dénommées en tant que telles) et n'entrent dans le cadre d'aucune nomenclature disponible couramment utilisée pour *A. cerana*.

Les données diffèrent de celles d'études antérieures (Fig. 1), parce que la résolution d'échantillonnage a été accrue et les lacunes de l'Uttar Pradesh et de la plus grande partie du Népal comblées. Les morphoclusters sont statistiquement distincts en ce qui concerne le début de l'essaimage de reproduction et ceci procure un certain degré d'isolement reproductif temporel entre les morphoclusters adjacents.

***Apis cerana* / génétique populations / morphométrie / Himalaya méridional**

Zusammenfassung – Morphometrische Analyse der *Apis cerana* – Populationen in der Region des südlichen Himalaya. Auf einem über 2200 km langen von Pakistan bis Myanmar reichenden Transekt durch das Gebiet des südlichen Himalaya wurden

Arbeiterinnenproben von *Apis cerana* Fabr. gesammelt. Ihre morphometrischen Eigenschaften wurden mit der Faktorenanalyse und mit der Diskriminanzanalyse einer multivariaten Analyse untersucht. Insgesamt wurden 3704 Einzeltiere von Völkern aus 64 Sammelorten in einer Sammeldistanzauflösung von 50 km untersucht (Tab. I). 22 der 55 Messungen waren für eine Trennung der Morphokluster ausreichend.

Die Morphokluster liessen sich, von West nach Ost, auf folgende Gebiete eingrenzen: I auf Kaschmir, II Himachal Pradesh, III Uttar Pradesh und westliches Nepal, und IV zentrales und östliches Nepal, Sikkim, Arunachal Pradesh, Bengalen und Nagaland (Abb. 2 und 3). Darüber hinaus waren Morphokluster III und IV jeweils in drei biometrische Gruppen unterteilbar: III (a) nordwestliches Uttar Pradesh (b) nordöstliches Uttar Pradesh und östliches Nepal und (c) westliches Nepal; IV (a) östliches Fuβgebirge des Himalaya in Sikkim, zentralem Arunachal Pradesh und östlichem Nepal; (b) Brahmaputratal und Khasihügel, (c) Nagaland, Manipur und Mizoram (Abb. 2).

Diese Ergebnisse wurden auf Grund einer Kombination von Daten erarbeitet, die bereits zuvor für 44 Orte publiziert worden waren (Verma et al., 1989, 1994; Singh et al., 1990), ergänzt durch Proben von 20 weiteren Orten, durch die bedeutende Lücken des Transekts durch den südlichen Himlaya geschlossen wurden. Nach ihrem statistischen Status entsprechen die hier aufgezeigten Morphokluster getrennten Subspezies (werden allerdings nicht als solche benannt) und passen nicht in irgendeine der derzeitig verfügbaren Nomenklaturen von *A. cerana*.

Die Ergebnisse unterscheiden sich von früheren Studien (Abb. 1) durch die dichtere Auflösung der Proben und die Auffüllung der Sammlungslücken in Uttar Pradesh und dem gröβten Teil von Nepal. Die Morphokluster unterscheiden sich im Hinblick auf den Beginn der reproduktiven Schwarmtätigkeit. Dadurch ergibt sich bis zu einem gewissen Grad eine zeitliche reproduktive Isolation zwischen aneinandergrenzenden Morphokluster.

***Apis cerana* / Honigbienen / Morphokluster / Populationen / südliches Himalayagebiet**

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