

Comparative morphometric studies between black and yellow strains of Indian honeybee - *Apis cerana*

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ABSTRACT: The study aimed to investigate the morphological differences between the strains of the *Apis cerana* honey bee revealed that there were no major variations with morphology of black and yellow morphs but the proboscis length and number of hamuli on forewing showed some differences. The black and yellow morphs are reproductively isolated and differ slightly in two morphological characteristics out of the 13 examined. The study establishes that without any geographic restriction, population at higher and lower elevations exhibited differences in these two morphological characteristics of honey bees.

KEY WORDS: Feral colony, variations, strains, proboscis, hamuli

Honeybees are valued for their contributions to mankind and its essential role during pollination of agriculture and horticultural crops is overwhelming. A special feature of the honeybee community is its explicit organismal focus and is social insects and lives together in nests or hives. The honeybee is remarkable for the dancing movements it performs in the hive to communicate information to its fellow bees about the location, distance, size, and quality of a particular food source in the surrounding area. Maa (1953) divided honeybees into 3 genera based on the morphological features of the honeybees, i.e., *Micrapis*, *Megapis* and *Apis* (European and Asian cavity nesting bees). Ruttner (1988) re-investigated and summarized morphometric information on the eastern cavity-nesting bees, which he considered as one species *A. cerana*.

And further grouped the *A. cerana* populations into four subspecies, Northern subspecies – *A. cerana*, Japanese subspecies – *A. cerana japonica*, Himalayan subspecies – *A. cerana himalaya* and Southern subspecies – *A. cerana indica*. Though *A. cerana* is a single species, Oldroyd *et al.* (2006) demonstrated that Indian cavity-nesting bees (*A. cerana*) are reproductively isolated between the two morphs, black and yellow strains. The classification of morphs was based on the first 3 tergites on abdomen. Also, two morphs thrive themselves in hill regions (black) and plain lands. Considering these studies Chethana *et al.* (2014) tried to check if these morphs are diverse at genetic level utilizing the four different mitochondrial genes. Recently Shanas *et al.* (2022) ensured that the yellow and black strains taken as the study group

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Table 1. Morphological differences analyzed with 13 different characters between 20 black morph and 20 yellow morph - *Apis cerana*

Characters	Black morph		Yellow morph	
	Range	Mean	Range	Mean
Body length	13.21-13.26	13.231+0.42	13.18-13.21	13.196+0.09
Head length	4.25-4.28	4.268+0.24	4.34-4.37	4.355+0.03
Head breadth	3.34-2.38	3.357+0.22	3.34-3.37	3.349+0.10
Proboscis length	5.45-5.49	5.469+0.11	4.80-4.85	4.283+0.14
Antenna length	3.95-3.98	3.96+0.09	3.93-3.96	3.942+0.05
Forewing length	3.95-3.98	7.89+0.05	7.79-7.82	7.802+0.02
Forewing breadth	2.64-2.67	2.65+0.15	2.58-2.61	2.594+0.08
Hindwing length	5.57-5.60	5.582+0.08	5.51-5.55	5.528+0.05
Hindwing breadth	1.55-1.58	1.564+0.12	1.51-1.55	1.529+0.05
Hamuli no.	16-17	16.5+0.05	19-20	19.4+0.10
Tibia length	2.79-2.82	2.802+0.14	2.76-2.80	2.776+0.9
Metatarsus	1.95-1.98	1.964+0.10	2.00-2.03	2.015+0.02
Abdomen length	3.72-3.76	3.737+0.09	3.82-3.85	3.832+0.03

are indeed one species (either *A. cerana*, *A. indica* or *A. karinjodian*). The present study aimed to find if there are morphological differences which categorize the *A. cerana* into black and yellow morphs.

Morphological studies were carried out to resolve differences between yellow and black strain of *A. cerana*. The feral colonies were selected for better understanding of the native bees. Samples of yellow morphs (n=29) were collected from the plain lands of Doddaballapur (800m elevation), and black morphs (n=25) from the hilly regions of Madikere (1392m elevation) in Karnataka state. The sampled bees were immediately stored in 70% ethyl alcohol for further study. Subsequently sample was transferred to fresh ethanol after two days. Twenty bees from each (black and yellow strain) colony were employed for morphometric analysis utilizing the techniques and few characters as illustrated by Ruttner (1978, 1988). It was not accurate way to infer the two different morphs with only a single morphological criterion to define or identify species and hence 13 morpho characters were considered

for studies. Therefore, feral black and yellow colonies sampled from their respective colonies were considered. A total of 13 characters were evaluated: body length, head length and breadth, antenna length, proboscis length, forewing length and breadth, hindwing length and breadth, number of hamuli on wing, tibia length, metatarsus length, abdomen length. The measurements were carried out using Motic Image Plus 2.0^{ML} microscope and a computer aided measuring system based on Motic multimedia software offered by China Group Co. Ltd. The prepared slides were stored and deposited in Department of Entomology, University of Agriculture Sciences, Bengaluru, Karnataka. The microscope was calibrated to determine the value of each division on the ocular micrometer. The obtained value of the division was used in measuring the size of the characters. The microscope was calibrated with three different sizes *i.e.*, 1X, 2X and 4X. In the studies to compare two different morphs of *A. cerana* 13 different morphological characters were analysed (Fig. 1, Table 1).

The morpho characters studied among 20 samples



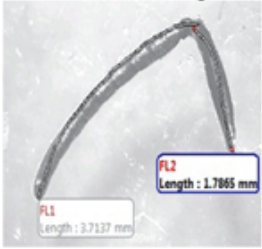
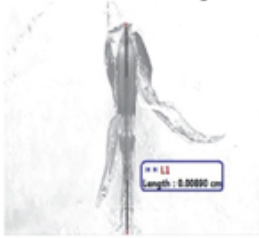
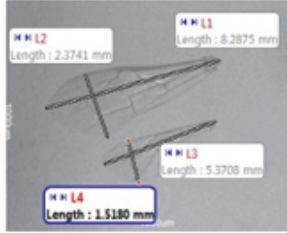
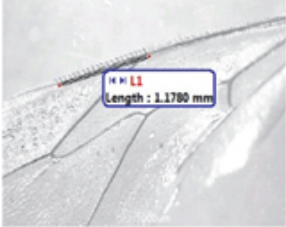
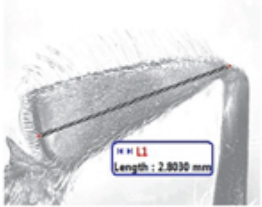
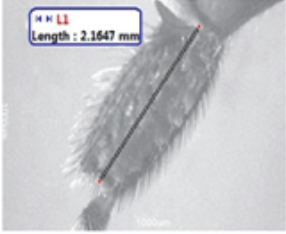
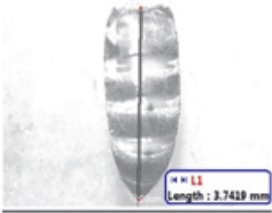
<p>1. Body length</p> 	<p>2 & 3. Head length & breadth</p> 
<p>4. Antenna length</p> 	<p>5. Proboscis length</p> 
<p>6 & 7. Fore & Hind wing length & breadth</p> 	<p>8. No. of hamuli on wing</p> 
<p>9. Tibia length</p> 	<p>10. Metatarsus length</p> 
<p>11. Abdomen length</p> 	

Fig.1 List of morphological characters considered for the study

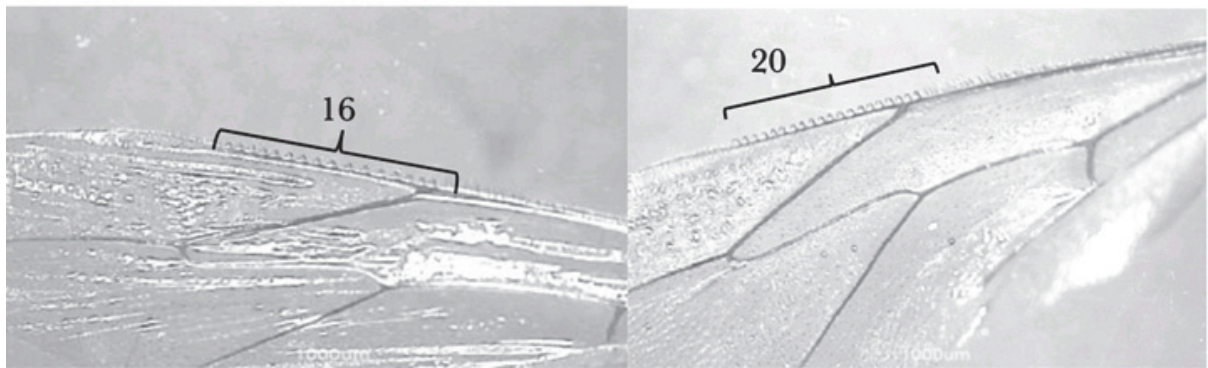


Fig. 2 Hamuli in black and yellow morphs

of each morph revealed the physical characteristics of the black and yellow morphs of *A. cerana*. Careful investigation revealed no major morphological differences between the black and yellow morphs, except for the proboscis length and the number of hamuli on the forewing showed some differences. The mean length of proboscis of the black morph was 5.469 ± 0.11 mm and yellow morph was 4.283 ± 0.14 mm and the mean number of hamuli on forewing of black morph was 16.5 ± 0.05 mm and yellow morph was 19.4 ± 0.10 mm. The other characters were quite similar, with negligible variations that did not contribute to significant differences.

During sampling of worker bees, an interesting observation was made that the tergites on the abdomen of the worker bee varied in its colouration, as discussed in earlier reports (Smith and Hagen, 1996; Oldroyd *et al.*, 2006; Bhaskaran, 2011). Usually, worker honeybee will have 8 tergites in which 5 are clearly visible and the rest are modified into the sting at the tip of the abdomen. The visible tergites were yellow colour in few bees and black in the others. The current study set out to determine whether the two different-colored bees were members of the same morpho-cluster or if any additional characters would distinguish them from one another. This issue raised the question of whether the two species were distinct from one another or if they were cryptic species or sister species. Morphological characteristics have created an interest towards two morphs showing more differences rather than tergites colouration (Kapil,

1956; Kshirsagar and Ranade, 1981). It was quite interesting to know that there were variations among two morphs for characters like proboscis length and the number of hamuli on the hindwing. Szabo (1990) who carried out in Sri Lankan bees, stated that the effect of temperatures might have caused these variations in the two different morphs.

The proboscis length of black strain was 5.469 ± 0.11 and yellow strain was 4.283 ± 0.14 mm. The length of proboscis in hill strain is longer than the plain bees. The present results were congruent with previous morphological studies (Kapil *et al.*, 1956; Mattu *et al.*, 1984a; Verma *et al.*, 1989; Verma, 1992) which indicated that nearness to sea influenced over the size of tongue of the bees might be a cause for such variations (Hepburn *et al.*, 2001a, b). This further may be ascribed to the kind of floral diversity at higher elevation would have made these bees to adapt with longer proboscis to collect the nectar and pollen during foraging.

Hamuli, which are hooks present on the anterior margin of the hindwing, are used for wing coupling and for fanning nectar to evaporate water content to produce honey. Their number perhaps may have role in the flight efficiency. In the present analysis, significant variation in hamuli number was evident (Fig. 2). The highest number of hamuli in the plain (yellow) bees was 20, and the least number in the hill (black) bees was 16, with mean numbers of 19.4 ± 0.10 in yellow bees and 16.5 ± 0.05 in black bees. The places of higher altitude >900 m had bees with a lesser number of hamuli when compared

with that of lower altitude <6000m above mean sea level. In other words, plain morphs had more number of hamuli than the hilly morphs. However, there was no similar trend in the number of hooks in the bees from north and south India (Kapil *et al.*, 1956; Jain *et al.*, 1967; Kshirsagar *et al.*, 1976; Mattu *et al.*, 1984a; Singh *et al.*, 1990). Present results were completely contrary to the earlier studies arising questions for the reasons behind this drastic divergence. It is predicted that extreme temperature conditions may have certain bearing on these parameters. Plain land would had led to high flight efficiency, increasing the distance of foraging rather than hilly region, resulting in a higher number of hamuli in plain bees.

The present study concludes that the black and yellow morphs are reproductively isolated and differ slightly in two morphological characteristics out of the 13 examined. However, extensive genetic studies are required to decide if the black and yellow strains are eco-races or subspecies of *A. cerana*. The elevation always has unique vegetation that attracts honeybees to fly higher, and these vegetations have flora that attracts bees which adapt to it with longer proboscis and the number of hamuli number to be higher for better flighting. The length of the proboscis is closely related to the deepness of the nectar glands of flowers recognized to be the best sources of food.

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