



Location specific morphological peculiarity of honey bee *Apis indica* in Amethi, region Uttar Pradesh, India: Revealization from an identification and characterization studied

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Abstract

Honey bees have an important place in agriculture and also suitable for environmental monitoring. There are four species occurring in India of which *Apis indica* is predominant bees found and domesticated in India. They usually build multiple combed nest in tree hollows and manmade structures. These bees can adapt to living in purpose-made hives and cavities. For the study on identification and morphological characterization of *Apis indica*, a total of 120 samples were collected from various sampling sites of District Amethi, Uttar Pradesh, India. These samples were identified with the help of standard taxonomic keys and various morphological characteristics recorded for morphometric analysis. After that these samples were preserved in 70% formalin solution for future reference. Honey bee (*Apis mellifera*) is being kept in different parts in the world. There are many practices which are done on honey bee colonies by beekeepers. Such practices (e.g. requeening and migratory beekeeping) lead to differences in the characteristics of honey bee colonies in the course of time. Morphological characters of honey bees can be measured to characterize honey bee populations and to be used as an indicator for productivity of honey bee colonies. The samples collected during various seasons revealed that many of the morphological characters such as length of flagellum, antenna, fore wing, third and sixth sternites and breadth of hind wing showed significantly higher values for the samples collected during summer and autumn seasons as compared to those collected in other seasons. The present study provides the important information on the morphological variation in Indian honey bee *Apis indica* in different seasons from the Amethi regions of Uttar Pradesh, India, which is an important parameter in racial investigation of honeybees. To characterize honey bee populations, the known method depends on the collection of random samples of honey bee workers from different hives and locations. However, there are different factors that can affect morphological characters. Thus, studying the stability degree of these characters is required to identify fluctuation levels within open populations of honey bees and to recommend the suitable method for its characterization.

Keywords: Indian honey bee, *Apis indica*, identification, morphometric analysis, Amethi U.P.

1. Introduction

The shape of organisms and their biological structures have been of scientific interest for centuries. This interest stemmed from the fact that most of the structures play significant role in the life and activity of most organisms. Also biological structures are the most conspicuous aspects of an organism's phenotype which normally provides a link between the genotype and the environment. Honey bees morphologic structures showed various adaptations for foraging, nectar collection, feeding the queen and the larvae, cleaning brood cells; removing debris, honey and pollen storage and rearing of larvae in cells made from wax secreted by the worker bees. Several works with *Apis mellifera* involving morphologic characters and weight showed that there is strong influence of the environment in the morphology of the same species [1]. There is often a positive correlation in some characteristics, for instance the length of the wing and altitude, the size of pollen basket and hind-leg and the size of honey stomach and honey production and storage. Such correlation can indicate the importance of the morphologic characteristics in the adaptation of individuals to the environment. The study area provides favourable ecological conditions and habitat for various economically important insect species and also

supports a good number of species diversity [1]. In [2] Saleem Ashamed, Rajneesh Tripathi presented the important information on the morphological variation in various forms of silkworm *Bombyx mori* in different seasons from the Amethi regions of Uttar Pradesh, India, which is an important parameter in racial investigation of this species of silk moth. Structures are adapted for grooming such as brushes, combs, scrapers amongst others and grooming behavior patterns are modified to permit manipulation and packing of pollen in the specialized transport structures. The addition and collection of nectar as well as its packaging permits the carrying of pollen of a great variety of sizes [2]. The common method for the characterization and classification of honey bee subspecies is based mainly on measuring honey bee wing characters, which were considered as strong tool. Various honey bee colonies, races and species were discriminated by employing morphometric analysis. Morphometric discrimination of *Apis mellifera* subspecies were widely reported in literature and several studies. The length of the proboscis was considered a very important character because it shows the geographical variability more accurately than all the other characters. As numerous and varied as studies on morphometric honey bee characters are, there exist a paucity of information

on morphologic characteristics of *Apis mellifera* for most ecotypes India.

The history of Apiculture dates back to several centuries, however the rich distribution and diversity of bees largely remains uncharacterized. The morphological and mitochondrial haplotype diversity across the range of *Apis cerana* does not provide a holistic view of the rich biological diversity in honey bees. Based on sequence analysis of mitochondrial DNA identified three major lineages of *Apis cerana*. However, exhaustive molecular characterization of honeybees needs to be taken up to resolve its rich diversity [4]. The rich pool of mitochondrial DNA provides adequate insights on the origin and the phylogenetic variation in honeybees over evolutionary time scales. Molecular studies on Asian honeybee-*Apis cerana* are carried out to a limited extent in few countries like China, Japan, Thailand, Philippines, Korea, Burma and India. The maternal inheritance and relatively rapid evolution of mitochondrial DNA has led to its widespread use of genetic marker for studies of maternal gene flow and the dynamics of hybrid zones. Over the past 2 decades, mitochondrial DNA studies have shed light on the biogeography of the Asian cavity nesting honeybee *Apis cerana*. Due to the relative ease of amplification and sequencing, in the last two decades the insect mitochondrial genome has become the most commonly used molecular markers for population genetics, phytogeography and molecular diagnostics. In India, based on morphological features, two “races” of *Apis cerana* are identified: a black ‘Hill’ morph, that is often said to live at higher elevation and a yellow “plain” morph found at lower elevations. The molecular studies of these two races were earlier identified in Indian samples. Within the eastern group of haplotypes, there are at least 5 lineages, one of which is the “Mainland Asia” group which has a broad range from India and Japan. One region of the *Apis* mitochondrial genome has proven to be particularly informative for intraspecific studies because the sequence does not appear to be subject to strong purifying selection and accumulate numerous base substitutions and insertions/deletions. Keeping the above in view, an attempt has been made in the present study to recognize geographic and genetic variability of *Apis cerana* within Amethi through molecular tools and characterize the relationships of *Apis cerana* with samples from various parts including adjacent regions of Amethi. This novel study would pave way for exemplifying the introduction of honeybees in India in evolutionary time scales and resolving the diversity in Indian honey bees [3]. Also, many other factors were suggested to have a key role in the shifting of worker bees from In-colony tasks to Out-colony tasks. Still more studies are required to elucidate the mechanisms influencing the shifting of the nurse bees to forager bees. After the change to foraging, a worker begins a new part of her life outside the colony in combination with different foraging tasks and interactions with the environment as explained in the next paragraphs.

2. Materials and Methods

2.1 The study Area

The study was conducted at Amethi, Uttar Pradesh, India. Honeybee samples were collected during the year 2014 to 2015 from different regions of Amethi representing different

climatic/vegetation zones 26.1541° N, 81.8142° E. Adult *Apis cerana* worker honeybees were collected from managed hives (from beekeeping centers) or feral colonies of local origin. A sample size of Yellow and Black strains varied between eight to ten samples of different strains of *Apis cerana* [1]. The collected bees were immediately transferred to vials with 95% alcohol and later stored at -80 °C until DNA extraction.

2.2 DNA purification and sequencing

The amplified DNA fragments were extracted from agarose gels and purified using the DNA purification kit “Easy pure” from Bio zyme. The primers used were the same primers used in PCR amplification and directly sequenced in “Big dye terminator version 3.1” cycle sequencing kit with sequencing machine—ABI3500xL Genetic analyzer. Then the data analysis of sequences was done using MEGA v5.4.

2.3 Preparation of floral calendar

Bee flora (Bee botany) of Indian honeybee was studied during the period 2009-13. The different pollinating plants were observed by bees. The list of bee past foraging plants includes field crops, vegetable crops, weeds, and ornamental and road side avenue trees [1].

2.4 Observations on bee foraging plants

Observations were made during different day hours. These were being visited by honey bees for collection of nectar or pollen or both. Such information is gathered for whole year. The floral calendars thus prepared will indicate honey flow and dearth period of the flora and the management of the colonies can be done accordingly.

2.5 Neighbour Joining tree

The NJ tree was quite similar to the DNA Parsimony tree, in which the ACMDK_COIY stood as an out-group. Amongst three haplotypes constructed, the Black and Yellow were separated from each other, but one haplotype had a Black strain along with two Yellow strains. The Neighbor joining phylogenetic tree of COI gene [2]. The distance matrix calculated using the NJ method amongst the strains (Black & Black and Yellow & Yellow) and between strains (Black and Yellow) is illustrated in Table 1.

Table 1: Illustrates the distance matrix amongst and between the strains

Combination	COI	Difference of distance
Apis cerana Black & Black	0.026087 to 0.088399	0.06230
Apis cerana Yellow & Yellow	0.004416 to 0.04664	0.04232
Apis cerana Black & Yellow	0.027003 to 0.89985	0.87293

3. Results and analysis

The result of the seven major morphologic structures measured in the honey bees of the study area are depicted by acronyms as: Head Length (HDL), Antenna Length (ATL), Proboscis Length (PBL), Thorax Length (TOL), Abdomen Length (ABL), Fore-wing Length (FWL) and Hind-wing Length (HWL).

Table 2: Morphometric structures measured in *Apis mellifera*.

Variables measured (mm)	Abbreviations
Head length	HDL
Antenna length	ATL
Proboscis length	PBL
Thorax length	TOL
Abdomen length	ABL
Fore-wing length	FWL
Hind-wing length	HWL

The results obtained for 7 external morphologic characteristics measured of honeybees were presented in Table-3. The mean value obtained for head length (HDL) was 4.46 mm, maximum and minimum values of 4.50mm and 4.43mm with standard deviation of 0.0090; ABL mean (5.63mm), maximum (6.24mm), minimum (5.533) and standard deviation (0.259). FWL had a mean of (9.542mm) and STD of 0.009 and 0.317. The standard deviation obtained for all the morphologic characteristics showed that there is slight variation within the features in Amberi study except for TOL whose standard variation show high variance (0.4153) within TOL feature of worker honey bees.

Table 3: Mean characteristic of 7 external morphological characters of honeybees in Amethi

Character	Mean(mm)	Max.(mm)	Min.(mm)	Standard Deviation
HDL	4.46	4.50	4.43	0.0090
ATL	5.371	5.383	5.3633	0.014
PBL	6.333	6.366	6.266	0.035
TOL	4.536	5.21	4.517	0.4153
ABL	5.6395	6.2433	5.5333	0.2598
FWL	9.542	9.556	9.533	0.009
HWL	7.905	7.916	7.896	0.013

4. Conclusions

The study examined the morphometric of *Apis mellifera* adaptation to the ecology, vegetation and climatic conditions in Amati. The data obtained for the seven morphologic characters demonstrates very little disparity in size measurement as values were close in all respects because the vegetation and climatic conditions within the state are not too distinct and the honey bee strains were also similar. However, the value of the external morphologic characters showed an increase during the wet than the dry season. It is imperative from the result of this study that beekeepers should monitor and be conversant with growth, development and adaptive traits so as to check the normal development taking place within the bee's population, such growth index as measurement of characters are pointers to adaptive radiation within the prevailing honeybee species of any area. Beekeepers for efficient bee management and production should be cautious of the kind of bee species used for their apiary establishment by having adequate record to properly monitor growth and development. The current study identifies the Western and Eastern group of *Apis cerana* in 12 populations of Amethi. *Apis cerana* in India occurs in two strains viz., *Apis cerana indica* (the yellow plain morphs) and the *Apis cerana cerana* (the black hill morph). The investigations involved characterization of honeybees through morphological and molecular studies. The morphometric microscopic

examination of the tergites of abdomen and mitochondrial DNA-COI gene characterization revealed the presence of two strains of *Apis cerana* in Amethi populations. The pilot study is of vital importance in establishing the genetic diversity of the indigenous honeybees of *Apis cerana* in the Indian sub-continent.

5. References

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