



## Protein profiling of *Apis* species (Hymenoptera: Apidae) adult worker honey bees from North-western region of India

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**ABSTRACT:** In *Apis*, biodiversity studies at genetic and molecular level were done to identify the proteins responsible for polymorphism in different species of *Apis* viz. *Apis florea* F., *Apis dorsata* F., *Apis cerana* F. and *Apis mellifera* L. from north-west region of India using SDS-Polyacrylamide gel electrophoresis (PAGE). Some species specific protein bands were obtained by SDS-PAGE technique followed by Commassie BB staining and silver staining. The present results revealed that the total content of proteins was highest in *A. dorsata* with highest number of protein bands unique to species followed by *A. mellifera*, *A. florea* and *A. cerana*. Most prominent were at 38.7 kDa and 35.3 kDa in *A. dorsata*; 46.4 kDa and 29.0 kDa in *A. florea*; 83.9 kDa and 40.4 kDa in *A. mellifera* and 61.0 kDa and 27.9 kDa in *A. cerana* respectively. Some species specific protein bands with silver staining were as 33.6 and 59.2 kDa in *Apis florea*; 57.2 kDa in *A. dorsata*; 49.6 and 63.6 kDa in *A. mellifera* and 36.1 and 38.8 kDa in *A. cerana*. Based on identification of these unique bands, it is concluded that the species with wide geographic distribution have large number of protein bands as compared to species with least distribution area. © 2016 Association for Advancement of Entomology

**KEY WORDS:** *Apis*, polymorphism in honey bees, gel electrophoresis, species specific bands

### INTRODUCTION

Honey bees are efficient pollinators and honey producers and also as wax, propolis and venom providers. The performance of these services varies from species to species due to their differences at the molecular level. Changes in the environment and exposure to different pollutants like poisonous gases, radiations *etc.* cause mutations at the genetic level and thus get reflected at the protein levels. Biodiversity can be studied at molecular level by analyzing the variations in number and type of proteins in one organism (Kumar and Kumar, 2013; Mestriner, 1969; Nunamaker *et al.*, 1984; Sheppard, 1988; Sheppard and Berlocher, 1984; Sylvester, 1982). SDS PAGE protein profiling has an

advantage that the degree of genetic correspondence between populations or taxa of a biological species can be quantified. Iftikhar *et al.* (2011) established biodiversity of honey bee species on the basis of physiochemical analysis of honey and suggested that in addition to the morphometric methods, molecular techniques should be used for phylogenetic studies. El-Bermawy *et al.* (2012) characterized the Egyptian, the Italian and the Carniolian subspecies of *A. mellifera* workers using protein, isozymes and RAPD-PCR. They have reported low level of polymorphism and Egyptian, Italian and Carniolan subspecies were distinguished on the basis of 18, 5 and 4 unique bands respectively. Surendra *et al.* (2011) studied the toxic potential of venom from three honey bee species

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*viz. Apis cerana, Apis dorsata and Apis florea* on bacteria and fungi by using SDS-PAGE and reported highest susceptibility of the pathogens to the venom of *A. cerana* followed by *A. dorsata* and *A. florea*. Priya *et al.* (2013) have reported odorant-binding proteins and pheromone-carrying proteins from sting and mandibular glands of *A. cerana indica* using GC-MS and SDS-PAGE profile. Sixteen volatile compounds in the sting and 19 in mandibular glands of the worker honey bee were reported. But SDS-PAGE protein profiling has not been used to characterize or establish biodiversity in the four species of honey bees found in north-west India. There is little published data from north western region of India. Some literature is available on venom glands, hypopharyngeal glands or larval stages of workers, drones or queen of any single species of honey bee. Similarities and differences in protein and enzyme patterning of the whole body homogenate of *Apis mellifera* have been studied in different populations in other countries (Ivanova *et al.*, 2011, 2012; Kandemir and Kence, 1995; Krieg and Marek, 1983; Lee, 1993; Li *et al.*, 1986; Markert and Moller, 1959; Moradi and Kandemir, 2004; Sylvester, 1976). There are very few reports of biodiversity studies on honey bees using molecular techniques from India, therefore the present studies were done to find polymorphism in the honey bee species, using certain proteins for their applicability as biochemical markers for inter specific differentiation of four species of honey bees *viz. Apis florea, A. dorsata, A. cerana* and *A. mellifera* found in north western regions of India.

## MATERIAL AND METHODS

### Study area and Sample Collection

Honey bees, were collected from north western region of India. Collection of samples was made by insect trapping net and forager bees were collected. From the region, 25-50 bees of each species were collected and were frozen at -20°C for biochemical analysis. The average individual body weight of honey bee was 27 mg of *A. florea*, 57 mg of *A. cerana*, 69 mg of *A. mellifera* and 117 mg of *A. dorsata* respectively.

### Preparation of homogenates

Honey bees of each species were weighed and homogenized with homogenizer, POLYTRON PT 2100 in Phosphate buffer saline (PBS)-pH 7 for 5 minutes to prepare 10% homogenate. It was centrifuged at 5000 g in a refrigerated centrifuge (REMI) for 10 min at 4°C. Supernatant obtained was used for further analysis.

### SDS PAGE profiling of Proteins

Protein content of the above samples was estimated by employing the method of Lowry *et al.* (1951). BSA (20mg/100 ml) was used as a standard. In order to calculate the protein content in the samples, a standard graph was plotted.

Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) was performed on homogenates of four honey species, by the method of Laemmli (1970). Equal amount of protein (15 µg) from the homogenate of the four species was loaded in the wells. 10% resolving gel gave the best resolution of proteins after staining with Commassie Brilliant Blue R-250 (CBB) and 12% resolving gel was used for staining with Silver stain as the latter stain is highly sensitive and visualize the proteins present in extremely low quantities which cannot be seen by the former stain. Molecular weight markers (MWM) were also run on the same gel and were labelled as protein ladder. Bromophenol blue was used as a tracking dye.

### Molecular weight calculation

To determine the molecular weight of each band obtained on gel, first a standard graph was plotted for the markers by taking  $R_f$  values of the markers on X-axis vs log molecular weight of respective standards on Y-axis. Best fit straight line graph was obtained as shown in Fig. 1. Then molecular weight of each band on the gel was calculated using the graph.

## RESULTS AND DISCUSSION

In order to ascertain variations at inter species level sample proteins were resolved on gel as shown in

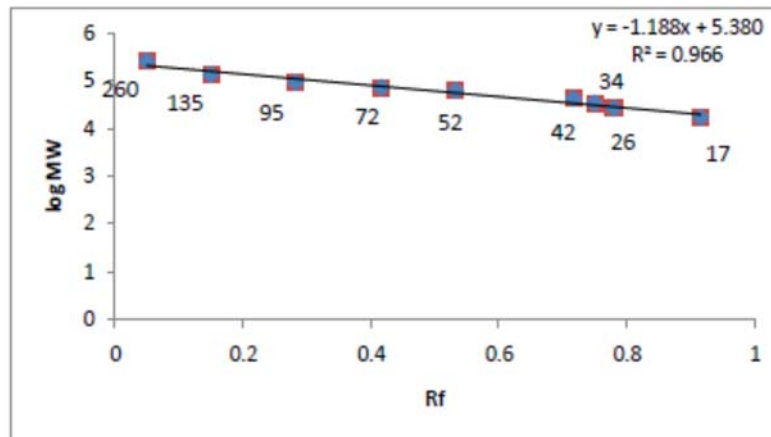


Figure 1.  $R_f$  vs log molecular weight (log MW) to determine the molecular weight of each band on the gel

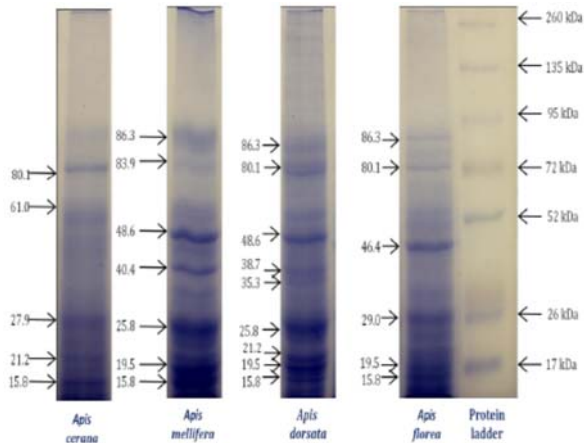


Figure 2. Protein profile of honey bee species on 10% SDS-PAGE [from left *Apis cerana*, *Apis mellifera*, *Apis dorsata*, *Apis florea* and marker]

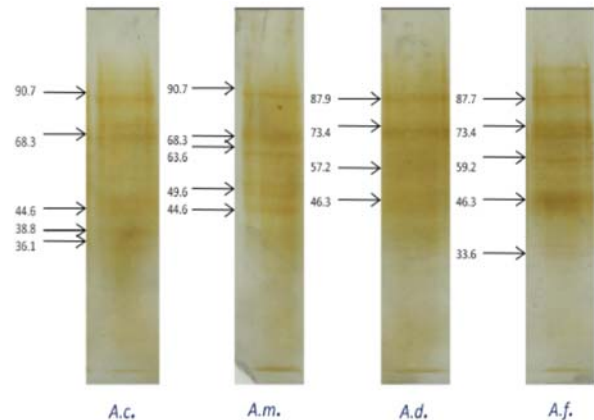


Figure 3. Protein profile of honey bee species on 12% SDS-PAGE [from left *Apis cerana*, *Apis mellifera*, *Apis dorsata*, *Apis florea* and marker]

Fig. 2 and 3 respectively. Protein profile of three native Indian species of honey bees *A. florea*, *A. dorsata* and *A. cerana* showed a common band of 80.1 kDa which was missing in the exotic species, *A. mellifera*. Protein bands of 19.5 and 86.3 kDa were observed in *A. florea* as well as in two other species of honey bees viz. *A. dorsata* and *A. mellifera*. *Apis mellifera* and *A. dorsata* exhibited similarity in banding pattern of proteins with molecular weights of 25.8 and 48.6 kDa. Rest of the Commassie stained protein bands were unique to different honey bee species. Protein bands common to all species studied (*A. florea*, *A. dorsata*, *A. mellifera* and *A. cerana*) were of molecular weights 15.8, 21.2, 25.8, 27.9, 29, 83.9

and 86.3 kDa respectively (Fig. 2). No such data on proteome analysis is available from India, however in studies reported by Surendra *et al.* (2011), common protein bands with molecular weight of 4, 5, 6, 7, 8, 9, 11, 15, 16, 31, 27, 30, 34, 35, 66 and 97 kDa were found in venom constituents of *A. cerana indica*, *A. dorsata* and *A. florea* using SDS-PAGE. During the present studies protein bands of 15, 21, 27, 61 and 80 kDa were observed in *A. cerana*. Priya *et al.*, (2013) also reported the presence of protein bands of 21 and 61 kDa in the sting and mandibular glands of *A. cerana*.

In the present studies protein bands with 15.8, 19.5, 40.4, 48.6, 83.9 and 86.3 kDa molecular weight

were observed in *A. mellifera* from Chandigarh. In previous studies, protein bands with MW 14, 21, 31, 45, 66, 97, 116 and 200 kDa have been reported in labial glands of *A. mellifera* (Sutherland *et al.*, 2006).

Using silver staining, molecular weights of proteins on SDS PAGE of the four honey bee species were calculated (Fig. 3). Three bands were observed to be common in *A. mellifera* and *A. cerana*. These had molecular weights of 44.6, 68.3 and 90.7 kDa. While *A. florea* and *A. dorsata* shared three protein bands of 46.3, 73.4 and 87.9 kDa, these were not found in *A. mellifera* and *A. cerana*. The silver stained protein bands unique to each species of honey bee were 33.6 and 59.2 kDa (*A. florea*), 57.2 kDa (*A. dorsata*), 49.6 and 63.6 kDa (*A. mellifera*) and 36.1 and 38.8 kDa (*A. cerana*). Sibebe *et al.* (2007) through silver staining of SDS-PAGE reported protein bands with molecular weight less than 25 kDa and more than 205 kDa in testes, seminal vesicles and accessory glands during different pupal stages of *A. mellifera* drones. In our results the protein bands differed in number, width and intensity among the different species studied thus exhibiting variation in number and quantity of proteins and enzymes, there by confirming polymorphism of proteins at interspecific level.

This paper can be considered as a study on protein profile of *Apis* species and polymorphism. The future implication include that the species specific protein bands can be isolated, sequenced, and searching similarities will give a clear picture of phylogeny which leads to biodiversity studies. Species specific protein bands of four honey bee species obtained from North-west region of India confirmed biodiversity at interspecific level and can be used as molecular markers for identification of species.

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