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Characterization of cavity dwelling honey bees using enzyme polymorphism

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Abstract
The most intensively studied of all insects are perhaps honey bees. Recognition of valid honey bee species has been difficult for several reasons. Most important reason has been the movement of honey bees all over the world for bee keeping which has resulted in their hybridization. Another problem is that scientists and bee keepers do not always use the same criteria for identification. While scientists are concerned with the biological parameters, bee keepers are more interested in behavioral traits. Even within a single species there are locally adapted populations called geographic ecotypes, which differ from each other in several morphological, biological, molecular and economic characteristics. Beside behavioral, morphological and cytogenetic evidence, electrophoretic data provide strong support for phylogenetic relationships among insects. *Apis cerana* and *Apis mellifera* are the Eastern and the Western cavity dwelling honey bees. Their habit of nesting in dark, enclosed spaces made it possible for man to domesticate them and to use them as a highly commercial industrial enterprise. *A. mellifera* is thought to have originated in the African tropics or Asia and colder European climates. The recent movement of bees by European settlers for bee keeping has resulted not only in worldwide distribution of *Apis mellifera* but has also led to some degree of hybridization between subspecies. On the other hand there has also occurred isolation of populations either by distance or by barrier giving rises to newer subspecies or races. To differentiate these honey bees on the basis of biochemical polymorphism alcohol dehydrogenase provided significant results and was observed to be an additional marker for the species.

Keywords: *Apis mellifera, Apis cerana,* biochemical polymorphism, systematics.

INTRODUCTION

Recognition of valid honey bee species has been difficult for several reasons. Most important reason is hybridization due to mobilization of honey bees all over the world and another problem is different approach of scientists and bee keepers for species identification. Even within a single species there are locally adapted populations called geographic ecotypes which differ from each other in several morphological, biological, molecular and economic characteristics. There are 8-10 species of honey bees. These include *Apis cerana* F. (Indian honey bee), *Apis florea* F. (dwarf honey bee*), Apis dorsata* F. (rock honey bee), *Apis laboriosa* Smith (large mountain honey bee), *Apis mellifera* L. (European honey bee), *Apis andreniformes* Smith (counterpart of *Apis florea*), *Apis koschevnikovi* Enderlin (the sister species of *Apis cerana*). All are native to India except *Apis mellifera*. Of all these species *Apis mellifera* and *Apis cerana* which are cavity dwelling bees are most important commercially as these can be domesticated and manipulated for economic gains.

Apis cerana (Eastern honey bee)

It is distributed throughout the Asia. It makes a series of parallel combs in dark spaces. This habit has facilitated its domestication and provides the base for traditional and commercial bee keeping in India.

Apis mellifera (Italian honey bee)

In wild the natural nesting sites of *Apis mellifera* are similar to those of *Apis cerana:* caves, rock cavities and hollow trees. The past three centuries have seen the introduction of this honey bee to all the habitable continents. It was introduced in India at Nagrota in 1960's, (Atwal and Goyal 1973). Outside Asia, bee keeping with *A. mellifera* constitutes an integral part of modern agricultural system, furnishing crop pollination services as well as honey and bees wax. With the variety of habitats, climatic conditions, and flora under which this species is found, it is not surprising that numerous sub-species have been identified in different regions. This bee species is so similar to *A. cerana* in morphology and behavior that they have frequently been considered as distant races of that species (Culliney 1983). A thorough evaluation of the systematics of oriental honey bees based on characters other than morphological and behavioral such as biochemical and molecular is very important and might lead to the identification of transition forms between *Apis mellifera* and *Apis cerana* (Winston 1987).

Biochemical and molecular records of some insects

The field of biochemical/ molecular technology has been exploited greatly in the last few years and many entomologists now wish to use this technology for the study of insects since it provides a new resolution at ecological and taxonomic level (Karp *et al,* 1998). Amino acid assay, protein markers, enzyme polymorphism, DNA

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analysis are techniques that have lately been used to understand and confirm insect systematics (Mestriner, 1969; Sylvester, 1982; Berlocher, 1984 and Sheppard and Berlocher, 1989). Such studies supplement the morphometric data and allow identification of populations even after some degree of hybridization brought about by the movement of insects from one region to another. Both protein and isozyme markers have revolutionized biological sciences and have enhanced the approach to molecular biosystematics. These markers are powerful tools that have facilitated the study of micro taxonomy. Beside behavioral, morphological and cytogenetic evidence, electrophoretic data provide strong support for phylogenetic relationships among insects.

Protein profiling

Separation of protein types (based on molecular weights) as distinct bands on polyacrylamide gel using the technique of SDS-PAGE provides important biochemical marker for systematic studies on insects (Avise, 1974 and Miles and Patterson, 1979). Monarch butterfly populations were separated using protein markers (Eanes, 1978). Kumar and Kamal (2003) studied the royal jelly profiles of honey bees and reported that the three species *A. cerana, A. dorsata, A. mellifera* could easily be differentiated by this technique. They suggested that the difference had systematic significance. Further, the technique could also be applied for separation of ecotypes within *A. cerana*. Protein profiles of the venom gland from different species of honey bees are found to be markedly different.

Isoenzymes

Isoenzymes analysis has become particularly prominent in systematic biology. The use of isoenzymes in entomological population biology has thus far been very limited and research involving something beyond the mere demonstrations of enzyme loci and allelic variants (allozymes) has been all but confined to *Drosophila* (Fox *et al,* 1971). However, the ground work is being laid for meaningful research in many insect groups. It is desirable that research involve diverse types of insects since *Drosophila* may provide a narrow and a typical view. Some representative studies on isoenzymes used to differentiate species, races and populations in insects include those of Trebatoski and Hayens, (1969), Warren and Berland, (1969), Narang and Kitzmiller, (1971) and Bullini and Coluzzi, (1972) on mosquitoes; Ogita, (1968) on houseflies; Tanabe *et al.,* (1970), Sylvester, (1986) Kumar *et al,* (2006) on bees and wasps and Johnson, (1971, 73, 76) on butterflies.

There are several methods for detecting isoenzymes. In order to use electrophoretic methods, it is necessary to be able to locate the enzyme on the gel by its specific activity. Currently about 80 enzyme substrates can be identified on gel after electrophoresis and then applying a color reaction to identify one of the reaction products (Shaw and Prasad, 1970, Gabriel, 1971a, b).

MATERIALS AND METHOD

Study material

Both cavity dwelling honey bees*, A. cerana* and *A. mellifera* were collected for developing and testing isoenzymes as markers to differentiate the two species.

Study area

A. mellifera was collected from apiary maintained by the Department of Zoology, while *A. cerana* was collected from the natural nests in Department of Biotechnology, Panjab University, and Chandigarh (India).

Sample preparation

For the experiment 5-7 worker honeybees were taken each time. The head, wings and legs of the insect were removed with the help of forceps. Only thorax and abdomen were taken for the preparation of sample. The material was taken in 1ml of phosphate buffer saline solution in a homogenizing tube and homogenized the tissue at ice-cold temperature by keeping the tube in beaker having chilled water. It was then centrifuged at 4 ° C at 2000 rpm for 5 minutes. The supernatant was taken for further experiments without disturbing the pellet. 20 µl was loaded onto each well in the gel. Electrophoresis was carried out in cold condition to avid heating effect. Electrophoretic run took 5 hours.

Staining of gel

It was carried out with specific substrate solutions for Mdh and Adh enzymes. It was incubated at 25 ° C and after appearance of bands gel was washed for photography for further study.

RESULTS

Malate dehydrogenase (Fig.1 and table-1)

Apis cerana

Three zones (Mdh-1, Mdh-2, and Mdh-3) of enzymatic activity were detected in the abdominal extract of this species. Each zone showed one band at mean Rf value of 0.868, 0.933 and 0.967. Two zones of enzymatic activity were detected from the thoracic extracts of this species. These zones showed two isoenzymes (Mdh-1 and Mdh-3) at mean Rf value 0.868 and 0.967.

Apis mellifera

Three zones (Mdh-1, Mdh-2 and Mdh-3) of enzymatic activity from the abdominal extracts were detected in the *Apis mellifera.* These zones showed three isoenzymes at mean Rf value 0.641, 0.815 and 0.956. Only two zones (Mdh-1, Mdh-2) of enzymatic activity were observed from the thoracic region in this species. These two zones showed two isoenzymes at mean Rf values 0.662 and 0.956.

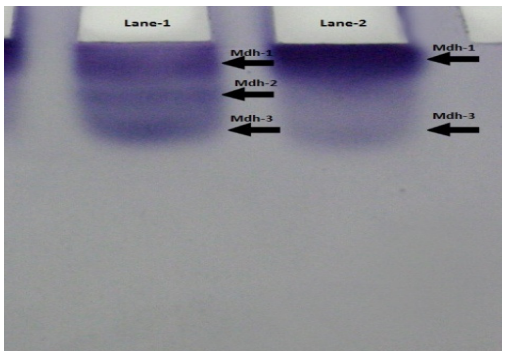

Fig 1. (Mdh)

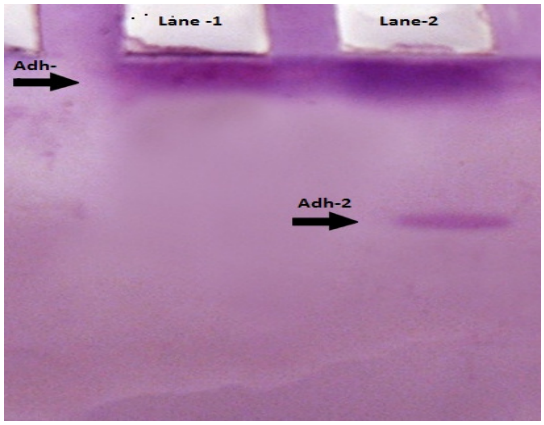

Fig 2 (Adh)

Alcohol dehydrogenase (Fig. 2 and table-1)

Apis cerana

One zone (Adh-1) of enzymatic activity was observed in the abdominal as well as thoracic extracts from the *Apis cerana.* This zone showed only one band at mean Rf value 0.615 for thorax as well as abdomen.

Apis mellifera

Two zones (Adh-1 and Adh-2) of enzymatic activity were observed from the abdominal extract and one zone (Adh-2) of activity was observed from the thorax extract in *Apis mellifera*. There were two isoenzymes with mean Rf values 0.681 and 0.943 for abdomen and 0.943 for thorax.

Table 1. Results of the Malate dehydrogenase and Alcohol dehydrogenase polymorphism in the *Apis cerana* and *Apis mellifera* through native discontinuous PAGE study.

Enzyme Studied	Species studied	Tissue extract	Zones of enzyme activity	Bands appeared	Rf value
Malate dehydrogenase (Mdh)	*Apis cerana*	Abdomen	3	Mdh-1	0.868
				Mdh-2	0.933
				Mdh-3	0.967
		Thorax	2	Mdh-1	0.868
				Mdh-3	0.967
	Apis mellifera	Abdomen	3	Mdh-1	0.641
				Mdh-2	0.815
				Mdh-3	0.956
		Thorax	2	Mdh-1	0.662
				Mdh-3	0.956
Alcohol dehydrogenase (Adh)	*Apis cerana*	Abdomen	1	Adh-1	0.615
		Thorax	1	Adh-1	0.615
	Apis mellifera	Abdomen	2	Adh-1	0.681
				Adh-2	0.943
		Thorax	1	Adh-2	0.943

DISCUSSION

The isoenzymic forms of Mdh were common to both species and are characteristic to the genus. Malate dehydrogenase demonstrated three distinct zones (Mdh-1, Mdh-2 and Mdh-3) of activity in the abdominal extracts of both *A. cerana* and *A. mellifera*. There were two zones of activity in the thoracic extracts of both *A. cerana* and *A. mellifera* these correspond to the isoenzymic forms Mdh-1 and Mdh-3. The banding pattern of Mdh was similar in the two species as also in the different tissue systems (abdomen and thorax) studied during the present investigations. The presence of all three Mdh isoenzymes as observed during the present study was also reported in the Norwegian *A. mellifera* (Sheppard and Berlocher, 1984). Mdh enzyme system did not therefore prove useful in characterizing the two species. This is in agreement with the findings of Nunamaker et al. (1984) who studied esterase and Mdh in the 3 honey bee species and concluded that Mdh could not be used for differentiating both bee species.

Apis mellifera showed in addition the presence of the form Adh-2 in the abdomen and thorax. The other cavity nesting bee *A. cerana* showed only one isoenzymic form designated Adh-1 in thoracic and abdomen region. So these cavity dwelling honey bees *A. cerana* and *A. melllifera* were distinct in showing different characteristic form of the enzyme (Adh). The polymorphism observed alcohol dehydrogenase during the present study provides additional marker for separating the two cavity dwelling species.

CONCLUSION

Present study embodies the results of investigations on biochemical molecular differentiation of cavity dwelling honey bees viz. *Apis cerana* and *Apis mellifera*. Native discontinuous PAGE technique was used for studying isoenzymes of Malate dehydrogenase and Alcohol dehydrogenase in these honey bees.

Alcohol dehydrogenase is polymorphic in dark nesting honey bees and can be used as a diagnostic tool for the differentiation of species. Thus it can be used as molecular marker for the differential diagnosis of cavity dwelling species of *Apis.*

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