Proteins Profiling as a Tool for Studying the Biodiversity of *PIERIS* SPP. (Lepidoptera)

Pooja Badotra, Neelima R. Kumar, Rakesh Chauhan

Abstract: ***India is a land of vast phsiogeographic variability. This subcontinent is rich in floral and faunal diversity. It is not surprising, therefore that the country is one of the richest in the world with respect to butterfly species. Butterflies are the foremost galore cluster of insects on earth that square measure simply recognizable by the overall public due their lovely colors and swish flight .They variate on the basis of sexual variations, individual, seasonal, geographical variations and races. These variations form the raw material for speciation and offer one of the most challenging aspects for the classification of these insects. The conventional morphological methods are of great help in separation of different species and genera. There is need to couple morphological, biological, behavioral and biochemical studies with available taxonomic information to separate insect taxa on the basis of discriminatory characteristics. Such supplementary characters come from biochemical and/ or molecular studies. Protein electrophoresis has emerged as a useful technique in population genetics and now a days been widely applied in systematics. Therefore, the present studies used this technique for the SDS-PAGE characterization and comparison of proteins in the both male and female of Pieris spp. (Pieris canidia indica and Pieris brassicae). It was interesting to note that after the comparison of the data, most of the protein fractions obtained in two species of P. canidia and P. brassicae were found to be common. And further, protein fraction were also common when they were compared within the same species male and female, confirming that these were the characteristic of the genus. So we can conclude that the proteins serve as good markers for the differentiation of species and also for the sexes in the same species.***

Index term: Biodiversity, Protein profiling, Pieris spp.

I. INTRODUCTION

Insects exhibit a diversity form and function important to the health of the ecosystem. They need perforate various and essential natural processes that sustain biological systems. The economic and ecological importance of insects makes them terribly valuable for studies of diversity. Butterflies are found in every part of the world wherever flowering plants are found, inhabiting even very high altitudes such as Artic, Antarctic, mountains covered with perpetual snow and glaciers. There are between 15,000 to 20,000 species of butterflies worldwide placed in the order Lepidoptera belonging to one of the two super families Hesperiodae (the skippers) and Papillionoida (all other butterflies). The order is of great economic importance. They have a double association with plants. In the caterpillars stage most butterflies eat and damage plant parts and are serious pests of economic crops.

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Pooja Badotra, Post Doctoral Fellow, IISER, Mohali, Ajitgarh, Punjab, India.
Dr. Neelima R. Kumar, Professor, Ph.D, Panjab University Chandigarh, Panjab, India.
Rakesh Chouhan, MSc Zoology, Panjab University, Chandigarh, Panjab, India.

In the adult stage they pollinate flowers, thus performing important ecological task. These insects are food for many birds, mammals and arthropods. Many species serve as indirect indicators of habitat quality as they are very sensitive to change in the environment and inform us about the presence or absence of particular plant species to which they are specific particularly during the larval stage. They also have esthetic and entertainment value for man.

Butterflies variate on the basis of sexual variations, seasonal variations, geographical variations and races. These variations form the raw material for speciation and offer one of the most challenging aspects for the classification of these insects. On account of the vast degree of color variations, the color patterns of the wings cannot alone be relied upon for the discrimination of different species and genera. A lot of taxonomic work has been done on butterflies by numerous workers all over the world based on external morphological characters such as labial palps, maxillary palp, wing venation, maculation, general coloration and armature of the legs [1], [2], [3] and [4]. There are however, numerous cases such as complexes and group of sibling species which need the help of other taxonomical features for their correct identification. Such supplementary characters come from biochemical and /or molecular studies. Although the butterflies are classified in two superfamilies Hesperiodae and Papilionoidae, these are sister taxa, so the butterflies collectively are thought to constitute a true clade. Some modern taxonomists place them all in superfamily Papillionoidae, distinguishing the skippers from the other butterflies at the series level only. A major new study [5] combining morphological and molecular data concluded that Lycaenidae and Riodinidae could all be strongly supported as monophyletic but the location of Pieridae within the pattern of descent was unclear, with different lines of evidence suggesting different conclusions. The family is of great economic importance with *Pieris* being a really damaging genus in agriculture. It is represented by the popularly known small and large whites present worldwide and serious pests of cruciferous crops. Because of the heavy infestation in cole-crops, this butterfly is also commonly known as the cabbage butterfly. Their host are plants belonging to the family cruciferae. The two species frequently encountered in North India are:-

A. *Pieris Canidia indica* Evans

It is commonly known as small cabbage white butterfly. The species is found in Himalayas, hills of Assam, South India and North India flying between the altitudes of 2500ft and 11000 ft. This butterfly has somewhat weak flight and keeps to low flying in open places and around bushes and shrubs. Wing expanse in 54-58 mm.
Butterflies are white in color.

Antenna is black, spotted with white, each with club distinct .Forewings white, dusted with black near base with black border along apex narrowly extended along tegmen, with a black spot in male and an additional spot in female. Undersurface is white dusted with black, spotted, its costal margins orange at base.

Butterfly keeps its wings vertically upward while resting on flower.

B. *Pieris brassicae* Gray

This is the common cabbage white butterfly. Ordinarily, it is a hill species found in Himalayas and hills of Assam flying between 3,000 ft and 12,000 ft. but in winters and early spring months it is also found in the plains adjoining the hills. Both male and female are white in colour and their wing expanse is about 72-78mm. Antenna is black in color, sparsely scaled with white, each with club gradual. Forewings are white, dusted with black scales at the base and along costal margins, more densely so in female, with a broad border and two black spots. Undersurface is white with rectangular black spot in female and a smaller black spot in both sexes. This butterfly has somewhat fast flight.

As stated earlier morphological methods alone are not sufficient today to elucidate the diversity in different groups of insects and newer techniques are being developed and employed by entomologists to confirm and establish the diverse taxa.

Separation of protein types (based on molecular weights) as distinct bands on polyacrylamide gel using the technique of SDS-PAGE and immunoassay provides important biochemical markers for systematic studies on insects [6,7,8] (Monarch butterfly populations were separated using protein markers [9] ,[10] and [11]. Hagen (1986) [12] used protein electrophoresis to differentiate members of the Papilio glaucus group and showed that there is some degree of hybridization occurring between P. glaucus glaucus and P. glaucus canadens.

II. OBJECTIVES

Since assessing biochemical variation by electrophoresis provides a simple technique for determining and confirming insect diversity, the present study was planned with the following objective-

To develop and standardize quick and authentic diagnostic tools for biosystematics studies on insect species.

III. MATERIAL AND METHODS

A. Study material

Pieries brassicae and Pieris canidia were taken for developing and testing biochemical markers for differentiating species.

B. Study Area

The study material Pieris brassicae (male and female) and Pieris canidia (male and female) was collected from the botanical garden of Panjab University, Chandigarh and adjoining fields (Fig.I and II).

Fig. I *Pieris brassicae* (Male) collected from Botanical Garden, Panjab University, Chandigarh.

Fig. II *Pieris canidia indica* (Male) collected from Botanical Garden, Panjab University, Chandigarh.

C. Technique employed

The standard technique of SDS-PAGE [6] was employed to study the protein profiles of Pieris brassicae (male and female) and Pieris canidia (male and female).

IV. RESULTS

The whole body extract of male and female Pieris brassicae and Pieris canidia were subjected to SDS-PAGE in order to determine the different types of protein fractions present.

A. *Pieris canidia* (male)

A total of 18 bands corresponding to different protein fractions were observed in Pieris canidia. The molecular weights of these proteins ranged from 9.50kD to 235kD and the distance travelled ranged between 6.5 to 0.3cm (Fig.III).

B. *Pieris brassicae* (male)

The Pieris brassicae male sample was also observed to be rich in protein types. 14 bands corresponding to different protein fractions were observed. Their molecular weights were determined by comparing with protein standards. These were found to be in the range of 8.00 kD to 235 kD and their relative distance travelled ranged between 6.80cm to 0.3cm.On comparison of the electropherogram of the males of the species, it was observed that a total of 11 protein types were common in the two species.

Three protein fractions with molecular weights of 174.28 kD, 18.75 kD and 8.00 kD were characteristics of P. brassicae, while the protein fractions with molecular weights of 68.75kD, 50.00kD, 38.00kD, 33.5kD, 28.50 kD and 22.50kD were present only in P. canidia.

D) *Pieris canidia* (female)

A total of 17 bands corresponding to different protein fractions were observed in Pieris canidia female. The molecular weights of these proteins ranged between 2.50 kD to 151.25 kD and the distance travelled ranged between 8.2cm to 0.6cm (Fig.IV).

E) *Pieris brassicae* (female)

The SDS-PAGE revealed 15 bands corresponding to different protein fractions. The molecular weights of these proteins ranged from 2.50kD to 220.kDand the distance travelled ranged between 8.2cm to 0.4cm.

Examination of the data revealed that as in the case of male, the female of the two species shared eleven protein types. The protein fractions with molecular weight of 220kD, 47.50kD, 38kD and 4.60kD were characteristics in P. brassicae (feamle), while 68.75kD, 28kD, 25.75kD, 17.50kD and 11.50kD were characteristic to female P. canidia. Comparison of the protein profiles of male and female of the same species revealed that there were 12 in case of P. brassicae. The fractions with molecular weights of 9.50kD and 66kD were typical to the males and with molecular weights 4.25kD and 2.50kD to the females in both the species.

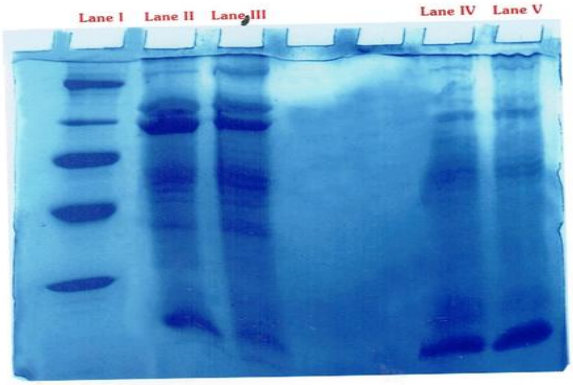

Fig. III: SDS PAGE of *Pieris canidia indica* (Male) (Lane II and III), *Pieris brassicae* (Male) (Lane IV and V) and their comparison with known standards (Lane I).

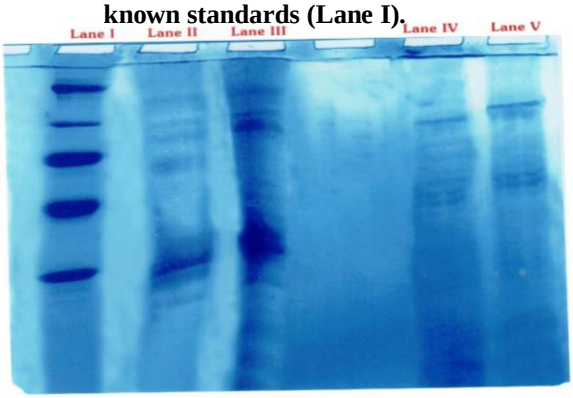

Fig. IV: SDS PAGE of *Pieris canidia indica* (Female) (Lane II and III), *Pieris brassicae* (Female) (Lane IV and V) and their comparison with known standards (Lane I).

v. DISCUSSION

Butterflies variate on the basis of sexual difference, seasonal variations and geographical variation races. These variations form the raw material for speciation and offer one of the most challenging aspects for the classification of the insects. Minor differences among allied species can be resolved by adopting a biosystematics approach. There is need to couple morphological, biological, behavioral and biochemical studies with available taxonomic information in order to select more reliable discriminatory characteristics for separating insect taxa. A lot of taxonomic work has been done on butterflies and other insects such as honey bees all over the world using modern taxonomic techniques [13], [14], [15], [16], [17] and [18].There were however, numerous cases such as certain complexes groups of sibling's species which need the help of additional taxonomic features for supplementing and augmenting available data/information. Such supplementary characters come from biochemical and/ or molecular studies. The field of molecular biology has today expanded greatly and many entomologists now wish to use this technology since it offers a new level of resolution for the study of insect ecological systems and systematics [19]. According to Berlocher (1984) [20] gel electrophoresis of proteins is by far the most widely used molecular technique in insect systematic. It has proven useful in all the three most important systematic tasks; species discrimination, species identification and hierarchical classification [21]. Berlocher (1984) [20] however, contends that electrophoresis is generally useful within the genera. This is the cue taken for the present studies. Protein profile of the butterfly's species revealed that there are more protein fractions in the male of Pieris brassicae and Pieris canidia india as compared to the female. This was in agreement with the observation of Gurleen (2006) [22] who estimated that the concentration of protein was higher in the male of Pieris brassicae and Pieris canidia indica being 1.03mg/ml and 0.63mg/ml as compared to the female (0.96mg/ml and 0.60 mg/ml respectively). There were 11 fractions with molecular weights of 235kD, 86.50kD, 69kD, 68.75kD, 66kD, 52.50kD, 47.50kD, 45kD, 44.50kD, 11.50kD and 9.50kD which were present in the male of both the speicies and can be regarded as characteristics to the genus. The protein fractions with molecular weights of 68.75kD, 28kD, 25.75kD, 21.25kD, 17.50kD and 11.50kD were specific to the male of Pieris canidia indica, while 174.28kD, 18.75kD and 8.00kD were specific to the male of Pieris brassicae. On comparing the protein types of the females of the species, it was observed that like in the male, there were 11 fractions which were present in both the species. The protein types with molecular weights of 68.7kD, 28kD, 25.75kD, 21.25kD, 17.50kD, 11.50kD were characteristic to Pieris canidia indica (female) while Pieris brassicae (female) had four characteristics fractions with molecular weights of 220.kD, 47.50kD, 38kD and 4.60 kD.

It was interesting to note that most of the species of Pieris canidia indica and Pieris brassicae were those which were also common between male and female of the same species further confirming that these were characteristics of the genus. Cook (1996) [23] has provided a ' Beginner's Guide to molecular markers' for entomologists who have some knowledge of molecular biology but are not especially conversant with the variety of markers presently available. Since the emergence of protein electrophoresis as technique in population genetics, it has also been applied widely in systematics (Burns, 1975) [24].The present results were therefore, indicative of the fact that proteins serve as good markers for the differentiation of species and also of sexes in the same species.

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FIRST AUTHOR PROFILE

Dr. Pooja Badotra, Post Doctoral Fellow, IISER, Mohali

RESEARCH EXPERIENCE:

Working experience with Hymenoptera from 2006-2010. Research experience with Hymenoptera w.r.t. biology, behaviour, systematics, molecular systematics, Phylogenetics, Ecology, Evolution physiology and pathophysiology. Experience of handling honey colonies, Colony management & manipulation.

Degree	Institu	Major /Specialization	Year	Division
Post Doc	Indain Institute of Science Education and Research, Mohali	Zoology	2017	Pursuing
PhD.	Panjab University, Chandigarh	Entomology	2013	------
M.Phil.	Panjab University, Chandigarh	Entomology	2007	1st
M.Sc	J.C.D.A.V College (Affiliated by P.U)	Zoology	2006	1st (3rd in PU)

RSEARCH PUBLICATION

Neelima R Kumar and Pooja Badotra (2008). Influence of Varroa jacobsoni Oudemans parasitization on some enzymes of carbohydrate metabolism in Apis mellifera L. worker brood. – Entomon 33(3):205-208.

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Pooja Badotra, Kusum Harjai and Neelima R Kumar (2014). Influence of Varroa mite parasitisation on associated microflora of Apis mellifera L. worker brood. Geobios 41: 18-23.
Academic Achievements: -
UGC- CAS meritorious student fellowship (2009-2010)
Poster and oral presentation (3rd rank) – 2nd Chandigarh Science Congress held on 14-15th March, (2008) at Panjab university, Chandigarh.
Second best poster presentation award at International conference on entomology held on 21-23rd Feb, (2014) at Panjabi University, Patiala.
Assisted in organizing the poster making contest jointly organized by Departments of Botany and Zoology DAV College, Sector 10, Chandigarh on 16th September, 2015.
Assisted in organizing the Debate Competition organized by Department of Zoology, D AV College, Chandigarh and sponsored by Vigilance Dept., State Bank of India, Sector 17-A, Chandigarh on 20th October, 2015.
Assisted in organization of the Inter- College Debate Competition organized by D A V College, Sector-10, Chandigarh in association with Chandigarh Senior Citizen's Association (Regd.) on 4th November, 2015.
Third best poster presentation award at International conference on entomology held on 3-5th Dec., (2017) at Panjabi University, Patiala.

SECOND AUTHOR PROFILE

Dr. Neelima R. Kumar, Professor (Re-employed) Ph.D. (Panjab University Chandigarh)

MAJOR ACHIEVEMENTS AWARDS

- Commonwealth Academic Exchange Fellowship
- Award of Honour in National Conference on Global Warming and Biodiversity Concerns, January 26-27, 2011
- Award of Honour in Global Extension Networking and Role of Women in Community Development, Oct 8-9, 2009
- Award of Honour in XV National Conference of the Biology Teachers, April 22, 2012
- Certificate of felicitation DST INSPIRE internship programme

RESEARCH HIGHLIGHTS

- Studies on poisonous glands of arthropods eg. centipede, scorpion, wasps, beetles, bugs.
- Development of low cost, eco-friendly hives for the native honey bee Apis cerana for better honey production, pollination and diversification of bee products for sustainable small scale bee-keeping.
- Studies on Royal Jelly: Structure of producing glands, biochemical nature in different species, standardization of method of production from the native honey bee.
- Evaluation of therapeutic potential of bee products such as pollen, propolis, bee venom.
- Development and standardization of queen rearing technology with native honey bee.
- Development and standardization of biochemical/molecular markers for studying honey bee diversity, geographic populations and ecotypes.
- Monitoring of insect pollinators of commercially important plants under the influence of the changing environmental scenario. Effect of electromagnetic radiations on honey bee behavior and physiology.

Academic Experience

- Teaching: 1981 till date
- Research: 1975 till date
- Publications: National–132, International–41, Bulletin–5, in Hindi-7, RadioTalks-5 TV Broadcasts-4

Projects

- Ministry of Agriculture: Development of technology for Conservation and Propagation of Apis cerana
- UGC: Studies on the Royal Jelly producing glands of honey bees
- Ministry of Env. & Forests: Biodiversity of Hymenoptera: Apidae • Dept. of Environment, Chandigarh admn. : Electromagnetic pollution from mobile phone towers: Effect on insect pollinators.

INTERNATIONAL EXPOSURE:

UNIVERSITIES/INSTITUTES WHERE WORKED/ VISITED

- University of Nairobi and Jomo Kanyatta University, Kenya.
- International Center for Insect Physiology and Ecology and National Bee Research Institute, Kenya.
- Forest Research Institute, University Pertanian and Universitty of Science, Malaysia. • Chulalongkorn University, Thailand.
- FAO and DST, Singapore.
- ICIMOD, Nepal

THIRD AUTHOR PROFILE

Rakesh Chouhan: MSc Zoology, Panjab University, Chandigarh.

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