



Amino-Acid Based Characterization of Three Pierid Species of Butterflies

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Article History

Received: 07/05/2016

Revised: 18/06/2016

Accepted: 22/07/2016

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Abstract

Butterflies have invited attention of numerous scientists who devised their own methods to identify and classify them under different categories. In this regard wing venation and genitalia have been extensively used. However, recently variations of intra-specific type have challenged its usage as a tool. This communication deals with a biochemical method of differentiating two species of genus Pieris of family Pieridae. Further the chromatograms of species under these genera are compared with that of a species referable to genus Catopsilia under same family. The amino-acid profile of the two species Pieris brassicae Linnaeus, Pieris canadia indica Linnaeus, Catopsilia pyranthe pyranthe Linnaeus has prepared using horizontal paper chromatography. Methodology involved and the results obtained have been discussed along with relevant figures to substantiate the text.

Key Words: Butterflies, Pierids, characterization, biochemical taxonomy aminoacids.

Introduction

The class Insecta of phylum Arthropoda is undoubtedly the most diverse group out of the entire animal kingdom. This class has numerous interesting orders, including Lepidoptera which is one of the largest out of all insect orders in terms of diversity. It has, attracted the enthusiasm of scientists all over the world. Rhopalocera and Heterocera are the two major groups that include butterflies and moths respectively. Butterflies are special because of their attractive appearance, beautiful wing maculation, reasonably good size, majestic flight and their sweet association with flowers. Butterflies sit in veliform position and assume planiform style only while basking in the sun whereas moths usually adopt planiform posture [1]

To accommodate such variable cluster of insects into delimitable groups mainly morphotaxonomy had been employed. The

keys to genera and species are mainly based on characters of wing venation. However, observations made by have raised doubts regarding its usage as important taxonomic tool to differentiate lepidopterous taxa [2,3,4]. This resulted in usage of a novel method in biochemistry in the current studies that is reliable and defines all possible similarities and variations among species. The motive of this method is to characterize free amino acids present in the haemolymph of butterfly species as also done by various scientists on other groups under class Insecta [5-19]. It involves the performance of certain simple and cost effective procedures wherein the amino acid comparison in two species of the same genera can be done so as to deduce their relationship. The results of the procedures are indicative of correlation between the three analyzed species viz., *Pieris brassica*, *Pieris canadia indica* and *Catopsilia pyranthe pyranthe* Linnaeus.



Fig. 1



Fig. 2



Fig. 3

Figs. 1-3: Adult of *Pieris brassicae nepalensis* (Fig.1) ; Adult of *Pieris canadia indica* (Fig. 2) Adult of *Catopsilia pyranthe pyranthe* (Fig.3) Linnaeus

Material and Methods: The butterfly fauna comprising of specimens of two commonly available species viz., *Pieris brassicae nepalensis*, *Pieris canadia indica* and *Catopsilia pyranthe pyranthe* Linnaeus (Figs.1,2,3) were procured from natural areas in and around Jalandhar city in the state of Punjab. Subsequently they were narcotized using Ethyl Acetate vapors and stretched immediately to avoid post mortal changes according to the technique given by Landry and Landry (1994)¹⁵. Free amino-acids were

extracted by method given by [16]. Circular Paper chromatography had been employed as per [20]. Solvent front comprising of n-Butanol : Acetic acid : Water (BAW); 40:10:15 was prepared and Whattmann Filter Paper - I was used as solid front. Standard amino acids were prepared with following proportions 2mg/ml 10 amino-acids in 10% isopropanol in N/10 molar HCl. The Relative Flow (Rf) values of both standards and test samples were calculated by the following formula:

$$R_f = \frac{\text{Distance travelled by amino-acid}}{\text{Distance travelled by liquid front}} \times 100$$

Result and Discussions

The analysis of chromatogram of three different species referable to family Pieridae revealed valuable information regarding component amino acids in their haemolymphs. *Pieris canadia indica* had presence of five amino acids and their Rf - values correspond to Ornithine, Glycine, Alanine, Tyrosine, and Methionine. In contrast to this another species *Pieris brassicae nepalensis* of the same genus also contained five amino acids that is Ornithine, Hydroproline, Serine, Threonine and Tyrosine [19]. On the other hand single species of genus *Catopsilia pyranthe pyranthe* of closely allied genus had prevalence of four amino acids which includes Lysine, Serine, Tyrosine and Leucine.

The two studied species of genus *Pieris* had amino acids Ornithine and Tyrosine common in them, whereas the remaining

three were different. This suggests their closeness to one another further corroborating their placement under the same genus. The third species belonging to a different genus *Catopsilia* showed prevalence of Tyrosine as one of the component amino acids which is also occurring in the two studied species of genus *Pieris*. The incidence of amino acid Tyrosine as common in the studied two genera is due to their pertaining to same family. Further, prevalence of nearly same number of amino acids in these species in spite of their belongingness to two different genera further strengthens the bond between them. Another important event was presence of Serine in *Catopsilia pyranthe pyranthe* otherwise only seen in *Pieris brassicae nepalensis*, suggesting more closeness in these two species as compared to *Pieris canadia indica*. [8,9]

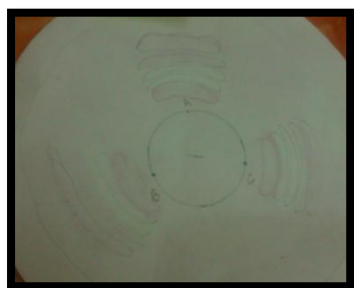


Fig 4



Fig 5

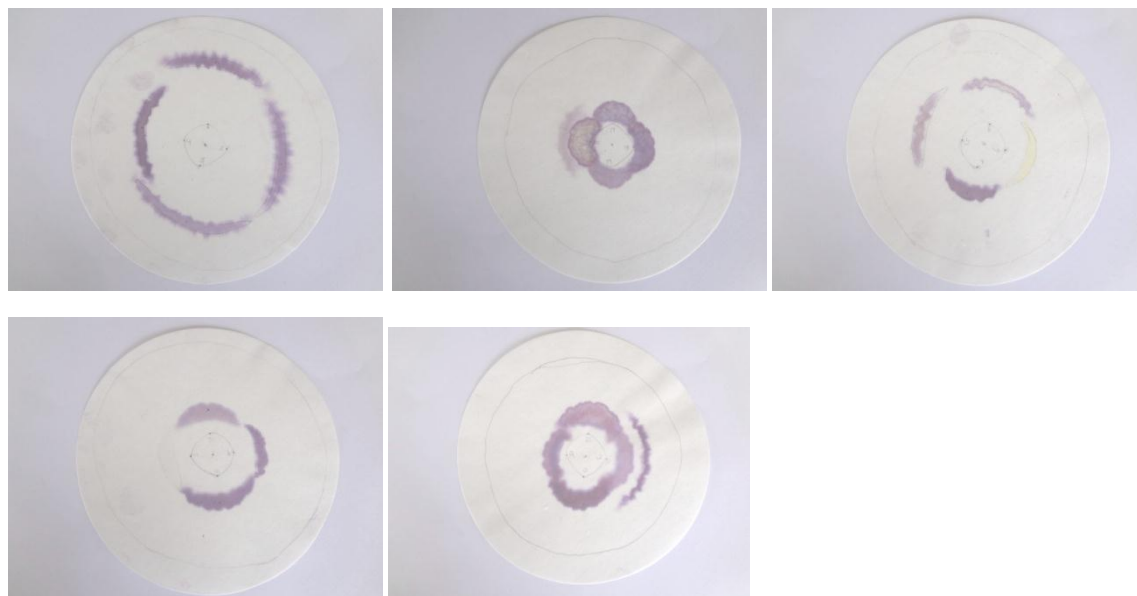


Fig 6

Chromatographs of *Pieris brassicae nepalensis* (Fig.4) ; *Catopsilia pyranthe pyranthe* (Fig.5); *Pieris canadia indica* (Fig.6)

The chromatograms therefore are good indicators congeneric status of two species, kept under *Pieris*. At the same time they also

deliver as a tool to confirm placement of genus *Catopsilia* as a close relative of genus *Pieris* under same family Pieridae.



Figs. 7-11: Chromatographs of Standard Amino acids

Acknowledgements

The authors are indebted to the Principal, Dr. BB Sharma of DAV College Jalandhar for his never ending support for conducting this research work. Further we are also thankful to Head, Dept of Zoology, DAV College, Jalandhar for providing necessary lab facilities and cooperation for doing the relevant experimental work. We also put on record the financial assistance provided in form of FIST sponsorship by DST, Govt. of India, New Delhi.

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