Original Research Article

Analysis of molecular data of some species of genus *Junonia* butterfly using RAPD technique

ABSTRACT

The present investigation was carried out to find out similarities and diversities between four species of genus *Junonia* belonging to Nymphalidae, the largest family of butterfly. The genomic DNA eluded from the species, *J. lemonias, J. alamana, J. hierta and J. orithiya* were screened by nine universal primers OPA-1, OPA-2, OPA-3, OPA-4, OPA-5, OPP-9, OPP-18, OPN-17, OPN-16 through RAPD - PCR. Primer OPN-17 did not produce any bands, whereas, discrete banding pattern was observed in remaining 8 primers. Primer OPP-18 distinctly highlights only species-specific bands ranging below 200 base pairs. Primer OPA-2 that produced significant banding patterns was taken into consideration for further analysis. The dendrogram was constructed by using binary data interpreted from RAPD gel image. Two clades were obtained, where in one clade taxa *J. hierta* and *J. lemonias* cluster as sister taxa, and in other clade taxa *J. alamana* and *J. orithiya* cluster as sister taxa. Investigation was supported by the Jaccard similarity and distance indices matrix and Principal component analysis. The interpretation from obtained dendrogram and distance matrix reveals that there is closeness within two species in their genetic makeup, whereas some genetic characters are expressed as species specific. Henceforth, these patterns produced by respective primers can be considered as diagnostic bands and may contribute to molecular markers for *Junonia* species identification. Thus, related evolutionary studies whenever be under investigation these markers will play a pivotal role in concluding the direction of evolution.

Keywords: Junonia; DNA; RAPD-PCR; Polymorphism; Dendrogram; PCA; Molecular Marker.

INTRODUCTION

Butterflies the most magnetic and captivating colourful creatures belongs to the largest order-lepidoptera under class- Insecta. Nymphalidae also known as brush footed or four-footed butterflies is the largest family of butterfly. Out of 18,000 species of butterflies found worldwide, 6000 species belong to nymphalidae and 521 nymphalidae inhabits in India [1]. Butterflies are key taxa for monitoring biodiversity as their population is susceptible to alternation in surrounding habitat specifically leading to loss of species with restricted geographical distribution [2, 3]. To keep a check on richness or destruction caused to environment there arises an urgency to conserve butterflies [4]. Molecular based work started by William [5] is since then continued and applied in various field like forensic investigation [6]; linkage map construction to state phylogenetic relations and morphological evolution among Nymphalidae sub family. Phylogenetic studies of taxa that exhibit adaptive phenotypic variation provide valuable insights into the evolutionary mechanisms driving the origins of biodiversity [7]. Butterfly species richness studied by Principal component analysis (PCA), is a powerful tool for analysing data that displays similarity between observation and of variables as points in spot maps [8, 9]. Main aim of investigation is to study species-specific genetic characters and closeness within two species in their genetic makeup supported by PCA.

MATERIALS AND METHODS

Collection and Identification of butterflies

Nymphalidae butterflies were collected from different region of Amravati by sweep netting and hand-picking method by observing their preliminary morphological data and later their taxa were identified by observing key characters as mentioned in Books of Indian Butterflies [1, 4, 10].

Preservation of specimen

The butterfly legs and thorax tissues were preserved in 70% alcohol [11] before post mortal changes.

Extraction of DNA

Genomic DNA was extracted from freshly collected leg tissues of butterflies by using Genetix DNAsure Tissue Mini Kit and stored at 4^0C for further use. Later it was quantified by using UV spectrophotometry and gel electrophoresis.

Amplification of genomic DNA through RAPD-PCR method

Genomic DNA were amplified in thermocycler gradient by using RAPD marker with primer ranged from OPA 1 to OPA 5, OPN 16, OPP 9 & OPP 18. PCR proves to be boon for molecular biologist as it is indispensable technique in biological and medical field and allows automated DNA sequencing, thus readily producing markers for further investigation [2]. Primers used with different percentage of base content are highlighted in Table 1.

Table 1. Primers used for RAPD-PCR profiling of Nymphalidae butterflies

Primers	Primer Sequence (5'to 3')	% Of GC content	Molecular weight (bp)
OPA 1	CAGGCCCTTC	70%	2964
OPA 2	TGCCGAGCTG	70%	3044
OPA 3	AGTCAGCCAC	60%	2997
OPA 4	AATCGGGCTG	60%	3068
OPA 5	AGGGGTCTTG	60%	3099
OPP 9	GTGGTCCGCA	70%	**-**
OPN 16	AAGCGACCTG	60%	**-**
OPP 18	GGCTTGGCCT	70%	**-**

PCR conditions were as follows [Table 2] and the amplicons gained were stored at 4°C.

Table 2. PCR conditions for amplification of Nymphalids DNA

Stage	***Temperature***	***Duration***
Pre-denaturation	94^0C	5 min
Denaturation	94^0C	1 min
Annealing	37.6^0C	1 min
Extension	72^0C	1 min
Final extension	72^0C	5 min
Total Cycles	**35**	

The PCR product was run at 100 volts on 2% agarose gel for 3- 4 hours. 10 µl amplicon along with 1µl of DNA loading dye were separated on gel plate stained with Ethidium Bromide and loaded with 1kb DNA marker. Bands on the gels were viewed under Imaging System Unit and photographed for further analysis.

Bioinformatics aspect

Bioinformatics is an interdisciplinary research area that is the interface between the biological and computational sciences. It is the application of computer technology to the management and analysis of biological data, as a result computers are being used to gather, store, analyse and merge biological data [12].

Application of MEGA-5 for analysis of brushfooted butterflies RAPD-PCR data

A computer program package called MEGA-5 is developed for estimating the evolutionary distance, reconstructing phylogenetic trees and computing basic molecular data. For the estimation of characters-based distance, some methods like Jukes and Cantor [13] distance, Tamura [14] distance, p-distance, UPGMA and Neighbor joining methods requires matrix of pair wise distance. MEGA is an integrated tool for conducting automatic and manual sequence alignment, inferring phylogenetic trees, mining web-based databases, estimating rates of molecular evolution, inferring ancestral sequences, and testing evolutionary hypotheses. MEGA is a multi-threaded Windows application. It runs on all releases of Microsoft Windows operating system [15].

Phylogenetic analysis

Phylogenetic trees are the most convenient way of visually presenting evolutionary relationships among a group of organisms. It can be drawn in various ways. The relationships established by phylogenetic trees describe a species evolutionary history and its historical relationships among lineages or organisms or their parts, such as their genes. In the construction of phylogenetic trees, the principle of minimum evolution or maximum parsimony is often used [16]. Different tree-building methods exist for the study of phylogenetic relatedness between DNA samples [17].

Principal Component Analysis (PCA)

It is a multivariate technique that analyse data table that describes inter-correlated quantitative dependent variables. It is mode of identifying pattern in data, and expressing the data to highlight similarities and differences [9].

RESULTS AND DISCUSSION

Preceding the amplification process of fragments of DNA, a data was scored in form of binary code, '1' for the presence and '0' for the absence of band for further RAPD analysis. Bands represents the genotypic characters. Nine primers were screened of that OPN 17 did not produce any bands. Remaining 8 primers showed different frequency of combinations of monomorphic and polymorphic bands. Whereas, Primer OPP-18 distinctly highlights only species-specific bands ranging below 200 base pairs. Primer OPA 2 producing significant banding pattern of the four *Junonia species* was taken into consideration for further investigation.

The similarity coefficient reveals maximum genetic similarity (0.3) between *J. alamana* and *J. orithiya* and minimum genetic similarity (0.066666667) between *J. lemonias and J. orithiya* [Table 3]. This data is strongly supported by Principal Component Analysis (PCA) dot map [Fig. 2], where *J. orithiya is* completely diverging out and is distantly located from *J. lemonias.*

Dendrogram [Fig 1] created based on binary molecular data shows two clades, where in one clade taxa *J. hierta* and *J. lemonias* cluster as sister taxa*,* and in other clade taxa *J. alamana* and *J. orithiya* cluster as sister taxa. Henceforth, all the above analysis reveals that there is closeness within two species in their genetic makeup, whereas some genetic characters are expressed as species specific. These patterns produced by respective primers can be considered as diagnostic bands and may contribute to molecular markers for *Junonia* species identification. Thus, related evolutionary studies whenever be under investigation these markers will play a pivotal role in concluding the direction of evolution.

Table. 3: Distance matrix based on RAPD-PCR of four species of *Junonia*

Species	*J. lemonias*	*J. alamana*	*J. hierta*	*J. orithiya*
J. lemonias	1			
J. alamana	0.1	1		
J. hierta	0.25	0.16666667	1	
J. orithiya	0.066666667	0.3	0.2	1

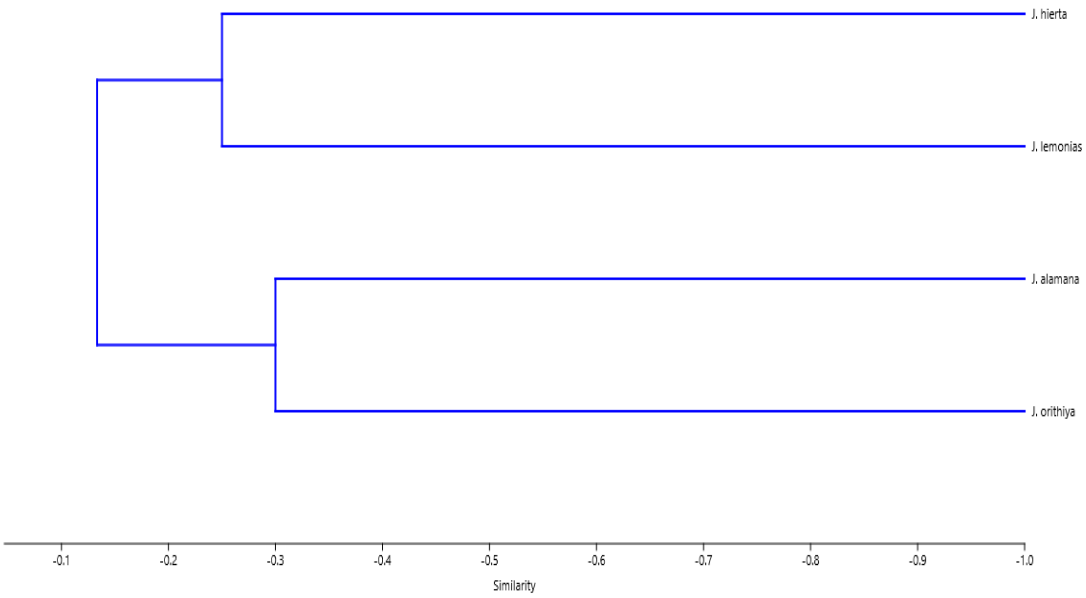

Fig 1: Jaccard Similarity coefficient of four species of *Junonia*

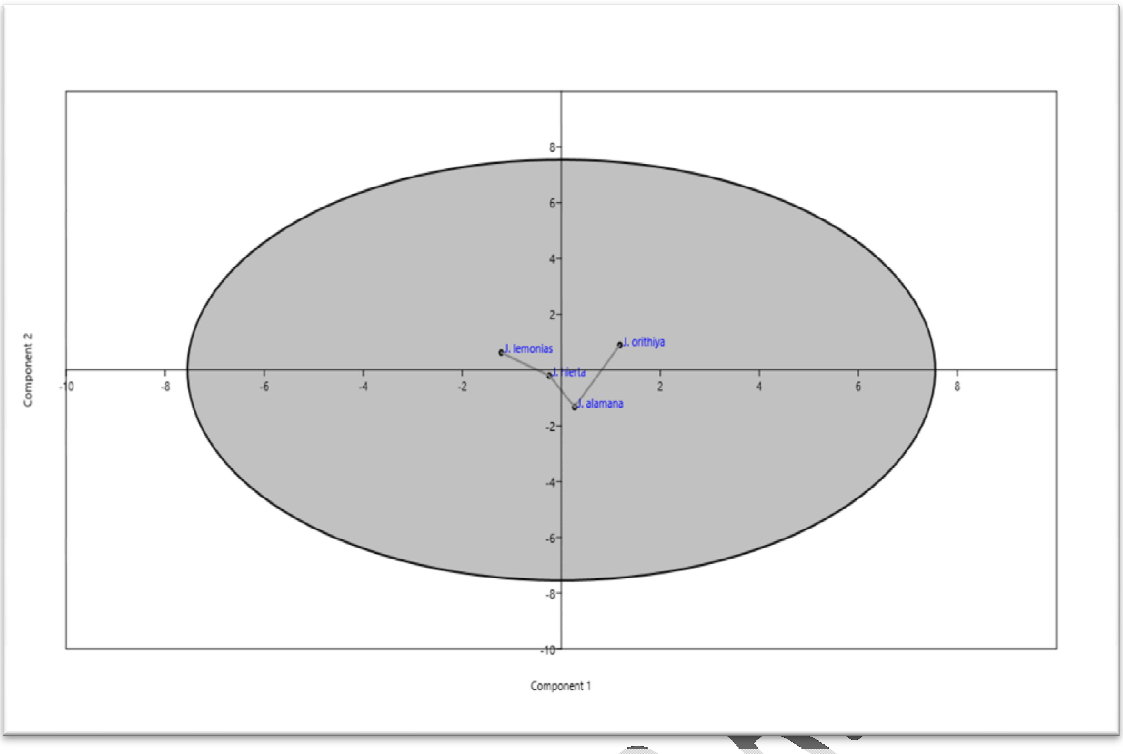

Fig. 2: Principal Component Analysis (PCA) of four species of *Junonia*

CONCLUSION

All the primers produced a large number of bands with different intensities and banding patterns suggested that the amplified fragments were repeated in the genome in varying degrees. Results suggested that the RAPD-PCR technique could provide a powerful tool to improve species identification and to better understand genetic variability and point where divergence is occurring within genus. Presence of species-specific bands among some species suggested the interspecific genetic relatedness between them.

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