Indian Journal of Ecology (2022) 49(2): 515-520
DOI: https://doi.org/10.55362/IJE/2022/3554

Manuscript Number: 3554
NAAS Rating: 5.79

Genomic Characterization of *Episyrphus balteatus* (Diptera: Syrphidae) from Outer and Lower Himalayas, India

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***Abstract*:** The present study deals with genomic characterization of a hover fly *Episyrphus balteatus* using Randomly Amplified Polymorphic DNA. RAPD-PCR analysis was carried out to compare genomic DNA of six geographically distinct populations of *E. balteatus* from Outer and Lower Himalayas using decamer oligonucleotide primers. The RAPD-PCR method detected a number of discrete DNA bands with different intensities. Ten random primers produced 39 bands in the studied populations out of which 25 bands were polymorphic. These polymorphic bands were used to differentiate the six populations of *E. balteatus*. PIC (Polymorphism Information Content) and RP (Resolving Power) values was 0.3312 and 6.06 respectively. Genetic similarity between studied populations ranged from 0.564 to 0.872. Among the studied population the lowest genetic distance (0.128) was observed between population P1 and P3 whereas highest (0.436) was observed between P5 and P6. These changes in DNA showed the geographical accumulation of variations with the course of time that leads to differential adaptability towards the environmental pressures. Dendrogram was prepared using UPGMA. Cluster analysis reveals various evolutionary adaptations of *E. balteatus* populations. P1 and P3 are classified as most related populations whereas P6 is relatively distant from the other five populations.

Keywords: PCR, RAPD, Genomic, Polymorphic, *Episyrphus*

Episyrphus balteatus, commonly known as marmalade fly is cosmopolitan and distributed throughout the world. Predatory dipterans of syrphidae family are considered to be the most important group of aphidophagus insects which acts as predators of aphid population. Larvae of syrphids rank as the major natural enemies for suppressing the aphid populations and thus act as bio-control agents(Nelson et al 2012). Although the genetic makeup of a particular population of a species remains same but the genetic response to the environmental stress or changes become altogether different if the populations inhabit different geographical regions or different climatic conditions. This can change the structure of DNA over a period of time and is sufficient to categorize different populations of a particular species. Identification of geographically distinct populations of *Episyrphus* may play an important role in pest management programmes.A variety of DNA based methods each with its own advantage and disadvantages have been developed over a period of time to identify cryptic species, subspecies or geographically distinct populations. Random amplified polymorphic DNA (PCR) has been widely used in dipteran families Culicidae, Muscidae, Callipharidae, Sarcophagidae as a potential tool to differentiate sibling and cryptic species, in strain identification and in genetic mapping(Skoda et al 2002, Malviya et al 2012). The randomly amplified markers appeared on different loci, gives a pattern which are unique to a single population and can differentiate the populations of different geographical regions. The degree of relatedness of these patterns shows the genetic differences among populations and it also indicates the extent of variation that may leads to formation of a new species from a population.In the study reported here we selected six populations of *E. balteatus* from different climatic regions of Middle or Lower Himalayas, Outer Himalayas and Shivalik ranges for RAPD-PCR analysis to study DNA polymorphism using ten RAPD oligonucleotide markers.

MATERIAL AND METHODS

Live specimens of six populations of *E. balteatus* belonging to family Syrphidae were collected from six different regions of outer and Lower Himalayas. These populations were named as P1 to P6 for six different regions with their altitudes as mean sea level (Table 1). Immediately after collection they were preserved in three different ethanol concentrations 70, 90 and 100% until DNA was extracted. Collections of *E. balteatus* were made in March-April, 2017 with average day temperature 30°C and 67% relative humidity. Leg muscles of adult flies were used for DNA extraction. DNA was isolated using following method of Skevington and Yeates (2000).

Isolation of genomic DNA: Genomic DNA was

standardized based on earlier procedures in terms of quantity, RNA contamination and DNA shearing. Tissue was mixed with 2% CTAB lysis buffer and incubated for 30 minutes at 65°C. After 5 minutes 300 µl of chloroform: isoamyl alcohol (24:1) was added and mixture was centrifuged at 10000 rpm for 3 minutes. The supernatant was removed and chloroform isoamyl alcohol step was repeated again. Further aqueous phase was collected into new tubes and 300 µl of chilled isopropanol was added. The tubes were kept for precipitation at -20°C for one hour and spun at 8000 rpm for 10 minutes. The supernatant was discarded and the DNA was washed with 200 µl of cold 70% ethanol. The DNA pellet thus formed was dried at 60°C for 30 seconds and was suspended in TE buffer (100 µl, pH 8.0 with RNAase 10 µg/ml)

DNA amplification and RAPD-PCR condition: PCR amplifications were done according to the protocol described by Williams et al (1990) with some modifications. Standardization of different components for PCR reaction was done by using the varying concentrations of template DNA, RAPD-PCR primers and Taq polymerase enzyme in 25 µl of reaction mixture (Table 2). Optimum concentrations of various components as worked out from standardization for a 25 µl reaction volume (Table 3) were consistently used in all the subsequent PCR-amplifications.

Three different DNA concentrations (10, 15, and 20 ng/µl) were tested and 10ng/µl was optimum for best amplifications of RAPD fragments. Polymerase chain reaction was carried out in Veriti 9-well thermocycler machine using the following cycling conditions: initial denaturation at 92°C for 5 minutes followed by 40 amplification cycles (30 seconds denaturation at 92°C, one minute annealing at 35°C and extension at 72°C for two minutes) and final extension at 72°C for seven minutes. The 10 primers, EB1- EB10 on *E. balteatus* samples to ascertain those suited for our level of analysis were tested (Table 4). Out of these, five generated

Table 3. Optimum concentrations of various components of PCR reaction mixture

Components	Volume in µl
Sterile water (mili-Q)	14.25
Taq polymerase (3U μl^{-1})	0.25
dNTP mix (2mM each)	2.0
DNA (10-20 ng μl^{-1})	1.0µl (10ng μl^{-1} final concentration)
RAPD primer	2.0
$MgCl_2$ (2.5mM)	3.0
Reaction buffer (10X)	2.5
Total	25

Table 4. Primers used for RAPD analysis of six populations of *E. balteatus*

Primer	Sequence
EB1	TGATCCCTGG
EB2	AGGGCGTAAG
EB3	CAGCCCAGAG
EB4	GTCCCGACGA
EB5	GGTGACGCAG
EB6	TGGGGGACTC
EB7	GTAGACCCGT
EB8	TGCGTGCTTG
EB 9	CTCTGGAGAC
EB10	TCTCCGCTTG

Table 1. Places of collection and their altitudinal range with in outer and lower Himalayas

S. No.	Location	Altitudinal range	Latitude (N)	Longitude (E)
P1	Doda (Bhaderwah)	1613 MSL	32° 58' 48.00"	75° 43' 12.00"
P2	Kathua (Nagri)	336-525 MSL	32° 22' 9.88"	75° 31' 31.40"
P3	Udhampur (Mianbagh)	756 MSL	32° 55' 27.52"	75° 08' 8.63"
P4	Poonch (Mandi)	981 MSL	33° 46' 12.00"	74° 05' 60.00"
P5	Jammu (Sehora)	327 MSL	32° 44' 8.48"	74° 52' 8.80"
P6	Rajouri (Shadra)	915 MSL	33° 14' 60.00"	74° 14' 60.00"

Table 2. Components of standardization PCR reaction mixture

Components	Variable PCR conditions
Template DNA (Episyrphus balteatus)	0.5-2µl (10-20ng/µl) DNA extract from single population was used in each PCR reaction mixture for determining the amount of template DNA for optimum amplification.
Primers	2.0- 5.0µl of RAPD primer (10nm solution) per reaction was studied for determining appropriate amount of primers in reaction mixture.
Taq polymerase enzyme	1-5 units of enzyme per reaction were used foe amplification of clear, distinctive bands of genomic DNA.

weak bands and five gave good result. The PCR products were separated on 1.5% agarose gels containing TE buffer and visualized using ethidium bromide. 100 base pair DNA size marker was used.

Analysis of PCR- RAPD results: Amplification products of each population were scored for the presence or absence of all bands identified. Ambiguous bands that could not be easily distinguished were not scored. The sizes of the RAPD bands were estimated by comparing with a 100 base pair ladder and recorded in a binary matrix that represent the presence (1) or absence (0) of a particular band. The binary matrix was used to estimate Jaccard's coefficient genetic similarity matrix and distance matrix within and in between different individuals (Jaccard 1908). A dendrogram was constructed based on the data of similarity matrix by using online (MAFFT version 7). Unweighted pair group method analysis (UPGMA). Polymorphism Information Content (PIC) was also calculated (Anderson et al 1993).

RESULTS AND DISCUSSION

During present investigation six geographically isolated populations of *Episyrphus balteatus* were studied for DNA polymorphism due to the effect of differential environmental pressure. The change in DNA sequences with the change in outer physical environment within a population can be easily detected through the use of RAPD-PCR technique. A number of discrete DNA bands with different intensities were shown by different primers tested. RAPD patterns were visualized and scored from gel photographs. Different primers used showed different species specific DNA bands and the level of amplification of some bands was not uniform. Out of 39 bands of genomic DNA formed by using ten decamer oligonucleotide primers, 25 bands were polymorphic in nature. These polymorphic bands were used to differentiate the six populations of *E. balteatus*. Maximum numbers of polymorphic bands were separated by primers EB2, EB3 and EB4 (Fig. 1b, c, d) and minimum number of polymorphic bands by primers EB5, EB7 and EB8 (Fig. 1e, g, h). EB1 and EB7 primer was successfully tested to define *E. balteatus* populations (Fig. 1a, g) where it separated three species specific bands (~1350, 1100, 500bp). EB2, EB3, EB4 (Fig. 1b,c, d) confirmed the possibility of an easy discrimination as it defined verydifferent RAPD profiles for the different populations of *E. Balteatus*. EB6, EB9, EB10 defined 2, 1, 1 species specific bands respectively (Fig. 1f, i, j).

The maximum polymorphic bands belonged to EB2 (4 bands), EB3 (4 bands) and EB4 (4 bands) with 100 percent DNA polymorphism and the minimum polymorphic bands belonged to EB7 primer (1 band) with 25 percent polymorphism. EB1 (3 bands), EB5 (1 band), EB6 (3 bands), EB8 (1 band), EB9 (2 bands) and EB10 (2 bands) showed 50, 33.33, 60, 33.33, 66.66 and 66.66 percent respectively. Primers EB1 and EB7 can be used to define the populations of *E. balteatus* for these primers produce some species specific DNA bands whereas primers EB2, EB3 and EB4 can be efficiently used to differentiate genetically different populations of *E. balteatus*. RAPD primers (monomorphic

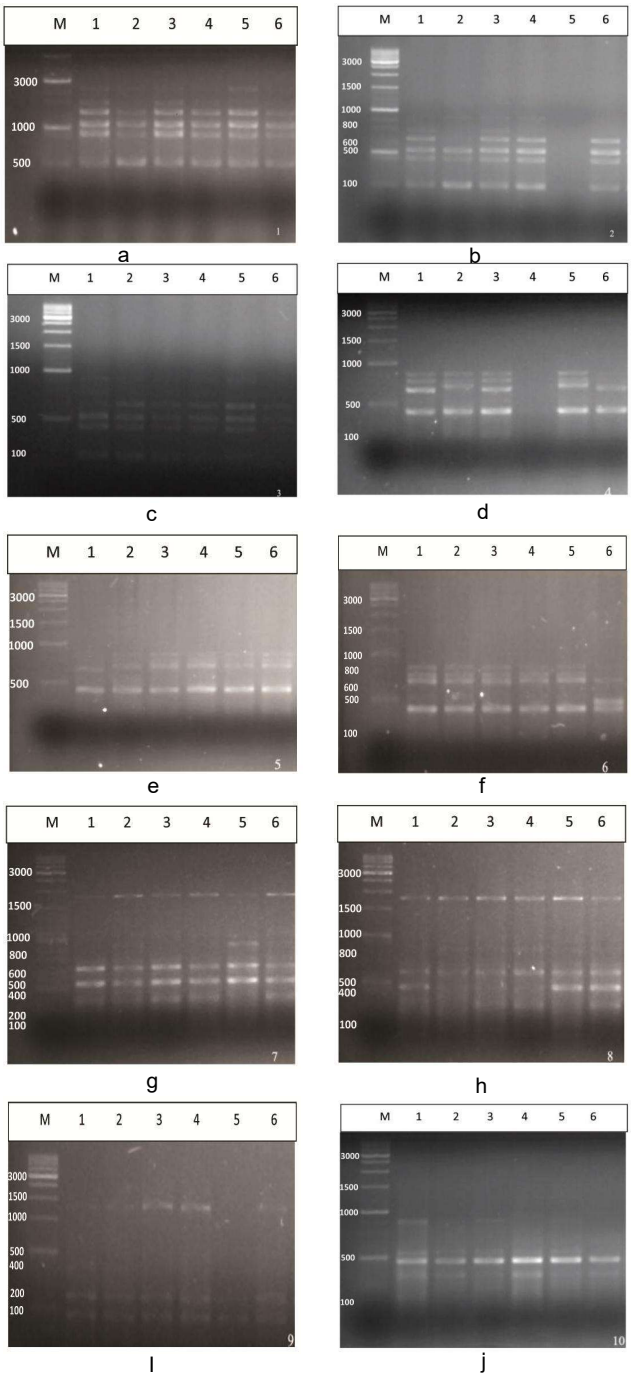

Fig. 1. RAPD-PCR banding pattern showing monomorphic as well as polymorphic markers in six geographically distinct populations of *E. balteatus*

and polymorphic) used to amplify the DNA from the individuals of the *E. balteatus* are mentioned here (Table 5 and 6 respectively). Fourteen monomorphic DNA bands (markers) were identified and these markers hold no practical value but are species specific in nature. Twenty five polymorphic markers identified hold enormous importance in molecular differentiation amongst the studied population. Different members in the six selected populations behaved differently with respect to the amplification of polymorphic markers. Use of these 10 decamer random primers identified genetic similarity index ranged from 0.564 between P5 (Jammu) and P6 (Rajouri) flies to 0.872 between (P1) Bhaderwah and (P3) Udhampur flies. High genetic similarity value is the indicator of genetic closeness of subpopulations. The present data indicates that P1 and P3 populations are most genetically related populations with lowest genetic distance of 0.128. The populations of Jammu (P5) and Rajouri (P6) region exhibited the highest genetic distance of 0.436.

PIC values, a reflection of allele diversity and frequency among the subpopulations ranged from 0.104 (EB5) to 0.547 (EB10). Presently used primers showed resolving power ranges from 3.66 (EB10) to 8.66 (EB1) (Table 7). The frequency of different allele for a specific primer among

Table 5. Different RAPD-PCR marker bands (monomorphic) observed in six populations of *E. balteatus*

RAPD Primer	Marker band (bp)
EB1	1300, 1000, 500
EB5	650, 400
EB6	700, 400
EB7	1850, 650, 500
EB8	1800, 600
EB9	100
EB10	450

Table 6. Different RAPD-PCR polymorphic marker bands amongst the populations of *E. balteatus*

RAPD-Primer	Marker band (bp)	*Episyrphus balteatus* population individuals					
		P1	P2	P3	P4	P5	P6
EB1	2500	1	0	0	0	1	0
	1500	1	0	1	0	0	0
	900	1	0	1	1	1	0
EB2	700	1	0	1	1	0	1
	550	1	1	1	1	0	1
	400	1	1	1	1	0	1
	100	1	1	1	1	0	1
EB3	650	0	1	1	1	1	1
	550	1	1	1	1	1	0
	425	1	1	1	1	1	0
	100	1	1	1	1	1	0
EB4	850	1	1	1	0	1	0
	750	1	1	1	0	1	0
	650	1	1	1	0	1	1
	400	1	1	1	0	1	1
EB5	850	0	1	1	1	1	1
EB6	800	1	1	1	1	1	0
	750	1	1	1	1	1	0
	500	0	0	0	0	0	1
EB7	900	0	0	0	0	1	0
EB8	450	1	0	0	0	1	1
EB9	1350	0	0	1	1	0	1
	200	1	1	1	1	0	1
EB10	900	1	0	1	0	0	0
	550	1	1	1	0	0	0

different populations of a single species suggests the amount of polymorphism shown by thepopulation. We can also relate these frequencies with the heterozygosity of respective populations. The frequency of different alleles can be used to calculate polymorphism information content (PIC) which is the information regarding the presence of polymorphic markers.

Statistical analysis of the data for genetic relatedness: All the monomorphic as well as polymorphic RAPD-PCR amplified bands were used to analyze genetic similarity between studied populations by using NTSYSpc version 2.2 software. The bands obtained were scored as '1' for presence and '0' for absence. The genetic similarity matrix was calculated using SimQual (Table 8). Among the studied population, the similarity of DNA bands ranged from 0.5641026 to 0.8717949. The lowest genetic distance of 0.128 was observed between populations collected from P1 (Bhaderwah) and P3 (Udhampur) regions. The populations of P5 (Jammu) and P6 (Rajouri) regions exhibited the highest genetic distance of 0.436.

UPGMA cluster analysis method was used to generate the dendrogram (Fig. 2). Both monomorphic and polymorphic DNA bands were taken into consideration for cluster analysis. On the basis of cluster analysis six populations based upon the genetic similarity data were classified into two main clusters A and B at the coefficient of GS 0.65. Group A consists of populations viz P1 (Bhaderwah), P2 (Kathua), P3 (Udhampur), P4 (Poonch) and P5 (Jammu). Group B consists of only one population P6

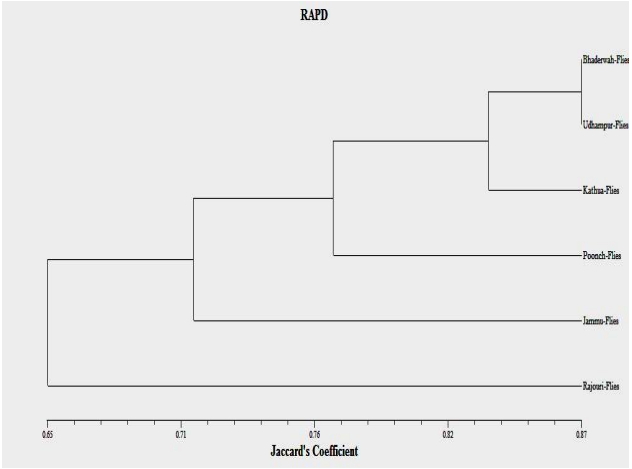

Fig. 2. Dendrogram showing genetic relatedness amongst six geographically distinct populations of *E. Balteatus*

Table 7. RAPD primers with the number of amplified bands, PIC (Polymorphism information content) and RP (Resolving power)

Primer	Nucleotide sequence (5'to 3')	PIC	RP	No of RAPD bands per primer	No of species specific RAPD fragments	Polymorphic bands (%)
EB1	TGATCCCTGG	0.39	8.664	6	3	3(50)
EB2	AGGGCGTAAG	0.37	6.33	4	0	4(100)
EB3	CAGCCCAGAG	0.307	6.664	4	0	4(100)
EB4	GTCCCGACGA	0.432	5.996	4	0	4(100)
EB5	GGTGACGCAG	0.104	5.666	3	2	1(33.33)
EB6	TGGGGGACTC	0.317	7.664	5	2	3(60)
EB7	GTAGACCCGT	0.243	6.332	4	3	1(25)
EB8	TGCGTGCTTG	0.25	5	3	2	1(33.33)
EB9	CTCTGGAGAC	0.352	4.666	3	1	2(66.66)
EB10	TCTCCGCTTG	0.547	3.666	3	1	2(66.66)
Total				39		25 (64.10)

Table 8. Genetic similarity matrix of six populations of *E. balteatus* derived from RAPD markers

	Bhaderwah	Kathua	Udhampur	Poonch	Jammu	Rajouri
Bhaderwah	1					
Kathua	0.7948718	1				
Udhampur	0.8717949	0.8717949	1			
Poonch	0.6923077	0.7948718	0.8205128	1		
Jammu	0.7179487	0.7692308	0.6923077	0.6666667	1	
Rajouri	0.5897436	0.6923077	0.6666667	0.7435897	0.5641026	1

(Rajouri). P1 (Bhaderwah) and P3 (Udhampur) populations were classified as the most related populations with a similarity percentage of 87% whereas lowest percentage of similarity occurred between P5 (Jammu) and P6 (Rajouri) populations with a coefficient value of 65%. Cluster analysis reveals various evolutionary adaptations of *E. balteatus* populations. P1 (Bhaderwah) and P3 (Udhampur) are classified as most related populations whereas P6 (Rajouri) population is relatively distant from the other five populations. Calado et al(2006) detected PCR-RAPD and PCR-RFLP polymorphisms in *Anopheles cruzii* (Diptera). They used seven primers for the comparisons within and among the populations of *Anopheles cruzii* during PCR-RAPD experiments. They calculated genetic distance among populations of *A.cruzii* varied from 0.0214 to 0.0673 suggesting that the individuals used in the analysis belong to a single species. Posso et al(2003) analyzed three populations of *Anopheles nuneztovari* (diptera) from Columbia to study genetic variation. They found a significant genetic distance (0.1131) between these populations. Variations among individuals were also significant (0.8869) by evaluation of these two parameters they concluded that three populations of *A. nuneztovari* are co-specific. Kaura et al (2009) characterized two populations of *Culex quiquefasciatus* using RAPD-PCR technique. Tyagi et al (2015) used RAPD DNA markers to differentiate the five sibling species of *Anopheles culicifacies*. Thetotal of 34 DNA bands was generated and these all are polymorphic in nature which can be used to differentiate between sibling species. The molecular weights of these bands were in the range of 2800 to 375 bp. They calculated average genetic distance between the species to be 0.58 ranging from 0.15 to 1.12. Silvester et al (2016) observed high intraspecific genetic diversity in populations of *Vibrio parahaemolyticus* along the southwest coast of India using RAPD-PCR. Tyagi et al (2012) studied genetic divergence in *Glycine max* genotypes from different environments.

CONCLUSIONS

The present research findings showed variations in DNA among six sub-populations of *E. balteatus* through the formation of 25 RAPD bands. The mean polymorphism percentage obtained was 64.10. The PIC value (allelic or intraspecific genetic diversity) ranged from 0.104 to 0.547. The genetic similarity of DNA bands ranged from 0.564 to 0.872. The lowest genetic distance of 0.128 was observed in case of P1 and P3 populations, indicating the most genetically related populations whereas the populations of P5 and P6 exhibited highest genetic distance of 0.436 indicating least relatedness. Present findings could be useful in population genetic studies as well as course of evolution among dipteran syrphid flies as less molecular data is available.

Received 27 November, 2021; Accepted 21 February, 2022

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