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Molecular genetic structure of fruit fly (*Bactrocera* sp) population of South Andaman revealed through RAPD-PCR analysis

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ABSTRACT

Fruit flies belonging to the family Dacinae, are biologically interesting and economically very important group of Diptera, as are known to infest a wide range of plant species especially fruits and vegetable crops. The present work was aimed for molecular profiling of fruit fly (*Bactrocera* sp) which is abundantly found in South Andaman. Though number of *Bactrocera* species has been reported to exist in these islands, *Bactrocera cucurbitae* is the most serious pest of cucurbit plants. *Bactrocera* sp is widely spread all over the world causing 85 -100 % damage to vegetable and fruits. Taxonomical diagnosis based on morphological characters could not discriminate them and all were found to be similar morphologically. During the course of this investigation, efforts were made to assess genetic similarities among these collections by using RAPD-PCR involving nine primers. The dendrogram of all primers depicted two major clusters which were having 60% similarity within as well as species. This shows a bright possibility of the use of RAPD-PCR in molecular profiling and identification of *Bactrocera* species of Andaman Islands.

Key words : *Bactrocera*, Taxonomical diagnosis, RAPD- PCR

Fruit flies belonging to the family Dacinae are biologically interesting and economically very important group of Diptera, as there larvae are known to infest a wide range of plant species causing severe loss to more than 125 species of fruits and vegetable crops. The term of fruit fly is two distantly related groups of flies (Alaoui *et al*. 2010). *Bactrocera* spp comes under the Drosophilidae and tephritidae. Tephritidae is another name in true fruit flies. The genus *Bactrocera*, with 350 to 375 species, is indigenous to Africa, the Mediterranean Region, the near and far East, Australia, and the Pacific (Samie and Fiky 2011). The damage caused by fruit flies raise the cost of fruit production and cause heavy losses to fruit production. The warm climates prevalent in Bay islands, India allows continuous cultivation of host plants ensuring continous supply of food for rapid multiplication. In India, more than 15 fruit flies of economic importance are reported from the genus *Bactrocera* (Diptera: Tephritidae). A survey of the fruit fly fauna in the Andaman and Nicobar islands revealed 11 species belonging to the genus *Bactrocera*. The Andaman and Nicobar islands include additional 6 species of dancines and also new host records for *Bactrocera (Bactrocera) carambolae,* and *Bactrocera albistrigata* (Ranganath and Veenakumari 1995). *Bactrocera zonata* is currently distributed in Sri Lanka, India, Pakistan, Thailand, Vietnam, Mauritius and Egypt (Liu *et al*. 2011, Jian *et al.* 2008). It has wide host range of more than 50 host plant species.

The development of DNA-based genetic markers has a revolutionary impact on animal genetics. Popular genetic markers in the entomological community are mitochondrial DNA, RFLP, RAPD, AFLP, Microsatellite, SNP and EST markers (Prabhakar *et al*. 2009). The application of DNA markers has allowed rapid progress in investigation of genetic variability, inbreeding, parentage assignments, species and strain identification and for the construction of high-resolution genetic linkage maps for insect species (Singh *et al*. 2011). Well-designed studies using these genetic markers will accelerate identification of genes involved in quantitative traits loci (QTL) for marker assisted selection (Zhang *et al*. 2010). RAPD-PCR analysis is used as a powerful method of understanding population genetic structure at molecular level in insect system (Jain *et al*. 2010). Keeping this in view, the present work was aimed for molecular profiling of fruit fly population, which is abundantly found in Andaman islands located in bay of Bengal and about 1000 km distant from mainland India.

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MATERIALS AND METHODS

During 2011- 2012, samples of fruit flies were collected from different places from the fields of bitter gourd from South Andaman with the help of fruit fly traps. After collection, samples were separated based on the morphological features. Four species of fruit fly were identified under island conditions. The samples were preserved at –80°C.

For the isolation of the total genomic DNA, a procedure followed by (Nishiguchi *et al*. 2002) was applied. The reaction mixture (20 µl) for PCR was composed by adding 50 ng template DNA, 1mM dNTP mixture (Bangalore Genie), 1 unit of Taq DNA polymerase (Bangalore Genie), 2.0 mM $MgCl_2$, 10XPCR buffer (Bangalore Genie) and 10 pmol of primer (Operon) for amplification. After preheating for 5 min at 94°C, PCR was run for 30 cycles. It consisted of a 94 °C denaturation step (1 min), 36°C annealing step (1 min), and 72 °C elongation step (2 min) in a Thermal Cycler Bio-Rad (CT-2000). At the end of the run, a final extension period was appended (72°C, 5 min), then stored at 4°C until the PCR products were analyzed. After amplification, the PCR products were resolved by electrophoresis on 1.2 % agarose gel and stained with ethidium bromide (0.5 µg/ml), followed by destaining and visualization under UV light. The gels were then photographed and raw gel images were recorded through Molecular Analyst Software/PC version 99.04). The DNA profile or fingerprint of each fruit fly population was recorded. The data was also scored for the presence or absence of the amplified fragments for all individuals (Nei and Li 1979). The data matrix was generated individual profile constructed using the following criterion: if a given amplified fragment was present in an individual, then it was assigned as '1' and when the fragment was absent, it was assigned as '0 '. Data were analyzed for genetic relationship using NTSYS- pc (Numerical Taxonomy System, Applied Biostatistics, New York, USA).

RESULTS AND DISCUSSION

Nine primers were screened on a group of selected individuals from each of the four *Bactrocera* species (*B. cucurbitae, B. dorsalis, B. zonata* and *B. invadens*) collected from South Andaman. Of the nine primers, five (OPK 05, OPK 06, OPK 10, OPK 13 and OPK 12) produced no fragments, and four primers (OPK 07, OPK 12, OPK 14 and OPK 15) showed polymorphic fragments among the *Bactrocera* sp. that were sampled under the PCR conditions chosen for this study (Table 1 and Fig 2, 3, 4 and 5). Four of the 9 primers showed polymorphic banding patterns and the number of bands that were generated per primer varied. For the final analysis, primers were selected, if they produced clearly resolved DNA banding patterns within and between populations. The method was found to be quite satisfactory and appreciably good quality DNA was isolated. The PCR protocol described in materials and methods resulted in reproducible patterns of amplicons by using specific combination of accession and primer. The sensitivity of RAPD-PCR technology has been found to change in accordance with experimental parameters. Keeping this in backdrop, the PCR reactions were performed exactly following the same protocol to ensure reproducibility. To use master mixes of dNTPs and primer from single source (Genei, Bangalore) utmost efforts were made. Nine decamer primers (Genei, Bangalore) were employed for RAPD-PCR amplification. Among the nine primers used, four showed amplification in the form of discernable bands in the agarose gel (1.2%). The results of RAPD-PCR are presented in Table 1. The 4 primers amplified 91 bands and majority of them were polymorphic in nature. The maximum number of bands was 33 amplified in (OPK 12) and least was 17 in (OPK 14).

Table 1 RAPD primers used for screening of *Bactrocera* sp. (*B.cucurbitae, B. dorsalis, B. zonata* and *B. invadens*) of south Andaman

Primer	Sequence (5'-3')	No.samples showing scorable bands \	Sample No. not showing any amplification informative bands [a]
OPK 07	AGCGAGCAAG	18	5,9,12
OPK 12	TGGCCCTCAC	33	
OPK 14	CCCGCTACAC	17	5,10,12
OPK 15	CTCCTGCCAA	23	12

[a]Tefers to bands absence in individuals from at least one stock

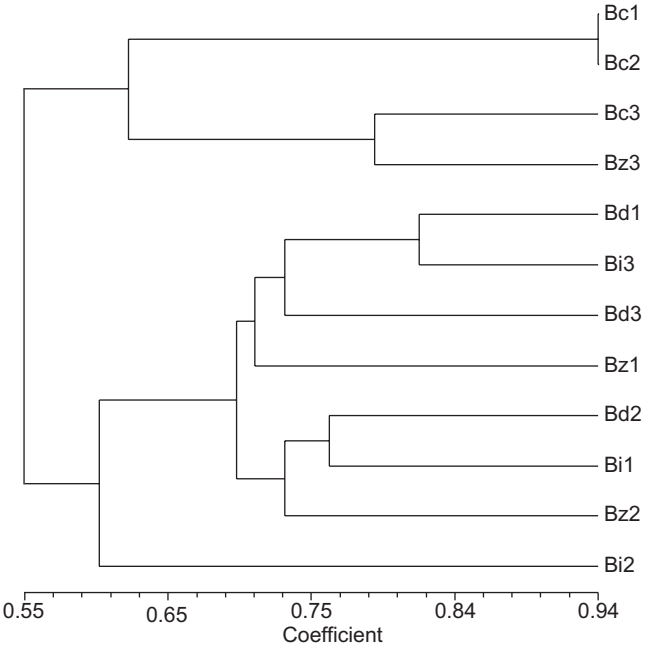

Fig 1 Dendogram showing cluster analysis using UPGMA based RAPD-PCR data of Fruit fly (*Bactrocera* sp.).

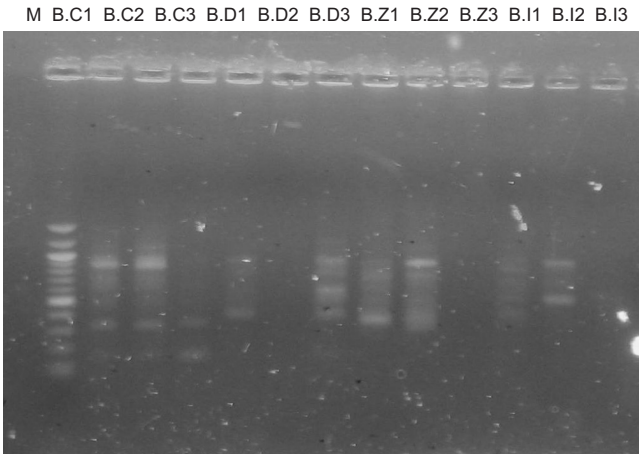

Fig 2 RAPD profile involving genomic DNA of fruit fly *Bactrocera* sp. of South Andaman Lane 1: 100bp, Lane (2-4) *B. cucurbitae*, Lane (5-7) *B. dorsalis,* Lane (8-10) *B. zonata,* Lane (11-13) *B. invadens* (OPK 07)

The sample 12 from *B. invadens* showed only one band and sample 1 of the *B. cucurbitae* showed twelve bands. By analyzing the banding pattern generated by the primers, we found inter and intra specific diversity in molecular level. The dendrogram showed a differentiation into two major clusters, assuming the populations are different (Fig 1). The upper cluster was comprised of only four individuals where the *B. cucurbitae* (Bc1 and Bc2) exhibited 100% similarities. The larger cluster was comprised of eight individuals with more than 70 % similarities with six sub clusters (Table 2). The sample *B. invadens* Bi2 was showing maximum diversity among all groups. Therefore, present analysis confirms this divergence between the populations that were sampled from the south Andaman.

RAPD-PCR is found to be the simplest and robust technique to distinguish *Bactrocera* sp. at molecular level. It

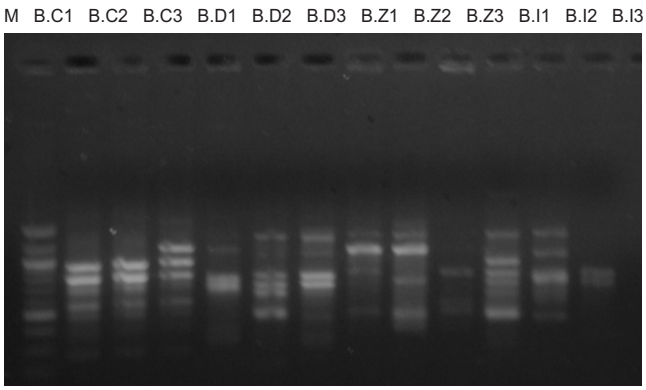

Fig 3 RAPD profile involving genomic DNA of fruit fly *Bactrocera* sp. of South Andaman Lane 1: 100bp, Lane (2-4) *B. cucurbitae*, Lane (5-7) *B. dorsalis*, Lane (8-10) *B. zonata*, Lane (11-13) *B. invadens* (OPK 12).

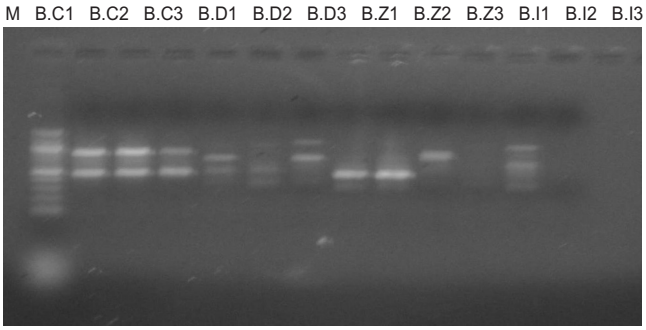

Fig 4 RAPD profile involving genomic DNA of fruit fly *Bactrocera* sp. of South Andaman Lane 1: 100bp, Lane (2-4) *B. cucurbitae*, Lane (5-7) *B. dorsalis,* Lane (8-10) *B. zonata*, Lane (11-13) *B. invadens* (OPK 14)

provides support to taxonomist, revealing information on the genetic constitution. The amplification pattern obtained by RAPD primers were considered to be ideal for identification of species. This technique has been widely used for systematics and population studies (Singh *et al*. 2011). Phylogenetic relationships among 24 *Bactrocera* sp. belonging to nine subgenera were inferred from DNA sequences of portions of the mitochondrial 16S rRNA, cytochrome oxidase II, tRNALys, and tRNAAsp genes. The evolutionary trend in male-lure response was evaluated in a phylogenetic context (Emtithal and Samie 2007). Currently, it has been proven that the use of molecular genetic data has a promising future in resolving and building phylogenetic relationships in the fruit fly population (Khamis *et al*. 2009). This method of finger printing is important since it is relatively easy to obtain valuable data allowing more introspective interpretation of diversity within the population (Jian *et al.* 2008).

The contribution from our work would eventually help to update the taxonomic status of *Bactrocera* genus especially

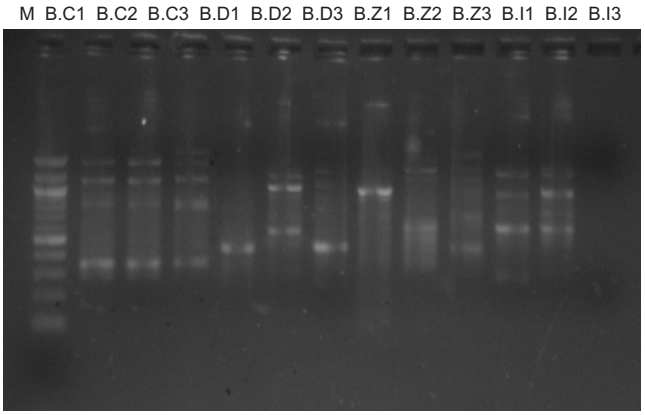

Fig 5 RAPD profile involving genomic DNA of fruit fly *Bactrocera* sp. of South Andaman Lane 1: 100bp, Lane (2-4) *B. cucurbitae*, Lane (5-7) *B. dorsalis,* Lane (8-10) *B. zonata*, Lane (11-13) *B. invadens* (OPK 15)

Table 2 Similarity index among *Bactrocera* sp. of south Andaman based on RAPD analysis

	Bc1	Bc2	Bc3	Bd1	Bd2	Bd3	Bz1	Bz2	Bz3	Bi1	Bi2	Bi3
Bc1	1.000											
Bc2	0.939	1.000										
Bc3	0.666	0.727	1.000									
Bd1	0.606	0.666	0.515	1.000								
Bd2	0.575	0.575	0.484	0.727	1.000							
Bd3	0.484	0.545	0.454	0.757	0.727	1.000						
Bz1	0.393	0.454	0.606	0.727	0.636	0.666	1.000					
Bz2	0.363	0.424	0.515	0.636	0.727	0.636	0.727	1.000				
Bz3	0.515	0.575	0.787	0.666	0.636	0.606	0.757	0.666	1.000			
Bi1	0.393	0.454	0.424	0.666	0.757	0.727	0.696	0.727	0.575	1.000		
Bi2	0.454	0.515	0.545	0.606	0.575	0.545	0.575	0.484	0.575	0.636	1.000	
Bi3	0.606	0.666	0.636	0.818	0.787	0.696	0.727	0.696	0.787	0.666	0.787	1.000

B. cucurbitae, B. dorsalis, B. zonata and *B. invadens*. Resolving the status of *Bactrocera* sp. *is* of interest to more than just the entomologist or taxonomist. Precise identification of species, development of diagnostic kits is important in addressing the quarantine regulations during import and export of fruits and vegetables. This is the first attempt to study molecular basis of species differentiation in fruit flies and will also serve as reference point for molecular basis of genetic variation within and among fruitfully populations of Andaman and Nicobar Islands.

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