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Anulin Christudhas
Department of Zoology, Madras Christian College, Tambaram-600059, Chennai, India.

Manu Thomas Mathai
Associate Professor, Department of Zoology, Madras Christian College, Tambaram-600059, Chennai, India.

Correspondence:
Anulin Christudhas
Department of Zoology, Madras Christian College, Tambaram-600059, Chennai, India.

Genetic variation of a migratory dragonfly characterized with random DNA markers

Anulin Christudhas, Manu Thomas Mathai

ABSTRACT
Polymorphism among individuals of dragonflies belonging to same genus and species was studied using molecular technique RAPD-PCR analysis. The RAPD banding pattern reflected the genetic diversity among *Pantala flavescens*. Reproducible and distinct polymorphic bands ranging approximately 200 bp to 2500 bp were generated with 5 RAPD primers. Operon c series primers OPC 7 and OPC 10 yielded unique bands of 1000 bp, 650 bp and 1100 bp which can be utilized for developing molecular markers for species identification specific for locations. The scoring pattern generated was utilized to construct the distance matrix using POPGENE 32 v1.31.

Keywords: Odonata, *Pantala flavescens,* RAPD, Molecular marker

1. Introduction
Dragonflies have fascinated human beings perhaps since the dawn of civilization for their variegated colors. They constitute a well-known and widely distributed insect group belonging to the sub order Anisoptera of the order Odonata. Odonates are probably the descendants of one of the most archaic insect group- protodonate which successfully flourished during the Upper Carboniferous and Permian periods about 255 million years ago. They have survived by developing extreme efficiency in the aerodynamics of their flight mechanism. Among the extant insects, dragonflies have gained importance because of their use in biological control programs. These predatory insects are now used invariably in toxicity testing because of their sensitivity to toxicants.

There are about 6000 species of Odonates worldwide. In India Fraser has documented 539 species and subspecies of Odonates. There are about 470 species belonging to 139 genera and 10 families within the political boundaries of India (Subramanian, 2009) [5]. *Pantala flavescens* (Fabr.) are wandering gliders, widely distributed and commonly found as groups migrating long distance. These strong fliers are predators feeding on flies during flight. The genetic structure of a species is affected by a number of evolutionary factors including mating system, gene flow, mode of reproduction and as well as natural selection (Hamrick *et al*, 1989) [1]. In spite of evolutionary changes the fossil dragonflies closely resemble the extant dragonflies. The present study was aimed to assess the genetic diversity among geographically isolated dragonflies. The hypothesis is that the population of dragonfly varies widely depending upon their habitat.

2. Materials and Methods
Insect Samples: *P. flavescens* were collected from five locations in India viz. Padmanathapuram (S1), Tambaram (S2), Puducherry (S3), Mizoram (S4) and Kerala (S5). The adult individuals were preserved immediately at -20 °C until DNA extraction for molecular characterization using Randomly Amplified Polymorphic DNA.

2.1 Genomic DNA extraction
Genomic DNA was extracted from single dragonfly of each population included in this study using protocol adapted from Saux *et al*. (2003) [3]. RNase A was added at a concentration of 10 µg/ml to the DNA sample and incubated at 37 °C for 1 hr. Equal volume of phenol:chloroform:isoamylalcohol (25:24:1) was added and mixed by inversion. The aqueous phase recovered after centrifugation at 10,000 rpm at 4 °C for 2-5 min was transferred with one-tenth volume of 3 M sodium acetate and 2.5 volumes of ice cold 99% ethanol. Samples were

incubated at -20 °C for 1 hr and centrifuged at 12,000 rpm for 10 min. Pellet was washed with 70% ethanol which was aspired by air drying. The pellet was redissolved in 50 µl of 1X TE buffer. The quality and quantity of DNA was analyzed by running each sample (with 2 µl of loading dye) in 0.8% agarose gel. The bands were visualized under the UV trans-illuminator with ethidium bromide staining.

2.2 PCR

RAPD-PCR analysis of dragonflies from different locations was performed using dragonfly DNA as template and five RAPD primers (Operon series) were screened. RAPD-PCR amplifications were performed in a total volume of 20 µl. Each reaction contained 50ng of genomic DNA, 2.5 U of Taq Polymerase, 0.2 mMdNTP's, 2 mM$MgCl_2$, 0.4 µM of primer (Operon Technologies Inc.) (Table1). The PCR reactions were carried out on a Eppendorf Mastercycler Personnel Thermocycler programmed at 94 °C for 3 min followed by 40 cycles of 94 °C for 30 sec, 37 °C for 1 min and 72 °C for 2 min, a final extension step at 72 °C for 2 min and stored at 4 °C. PCR products were separated in 1.5% agarose at 50 Volts with 1X TAE buffer. Gels were stained with ethidium bromide and products were visualized in a UV Gel documentation unit and photographed.

Table 1: The Oligonucleotide primer sequences used in RAPD technique.

Sr. No.	Operon	Sequence
1	OPC7	5′-GTCCCGACGA-3′
2	OPC9	5′-CTCACCGTCC-3′
3	OPC10	5′-TGTCTGGGTG-3′
4	OPC17	5′-TTCCCCCCCAG-3′
5	OPC20	5′-ACTTCGCCAC-3′

2.3 Analysis of RAPD-PCR profiles for genetic relatedness

To calculate the pair-wise differences matrix among the five dragonflies all the individual bands of different dragonflies from all five locations in RAPD banding profile were scored as presence (1) or absence (0). The statistical analysis of genetic variations between natural populations was performed using POPGENE 32 (Version 1.31) software (Yeh *et al,* 2000) [7]. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram was constructed with MEGA 4 (Tamura *et al,* 2007) [6] using Nei's unbiased genetic distance.

3. Results

3.1 Characteristics of bands generated by RAPD

Initially 5 RAPD primers were tested. For the second stage only two most informative primers which amplified scorable and reproducible DNA bands (with annealing temperature at 37 °C) were amplified for the analysis of 5 populations. The selected primers generated DNA bands with size ranging from 200 bp-2500 bp. The number of bands generated by individual primers was 10-11, being 10 for OPC 7 primer and 11 DNA bands for OPC10 primer. The total number of Polymorphic DNA bands was17, out of which OPC 7 generated 8 and OPC 10 generated 9 bands (Figure1). The Primers amplify a constant RAPD marker shared by the dragonfly species, *P. flavescens*.

Analysis of the RAPD profiles showed that the percentage of polymorphic loci of *P. flavescens* was less, suggesting a low genetic diversity. Eventually, an intraspecific marker has been identified and the population specific bands were documented for the following populations: S3 (1 band at 650 bp with OPC10 primer) and S5 (2 bands at 1000 bp with OPC7 and 1100 bp with OPC10). Thus, the markers obtained from the study, low expense and efficiency confirms applicability of the technique in studying Odonate population genetics.

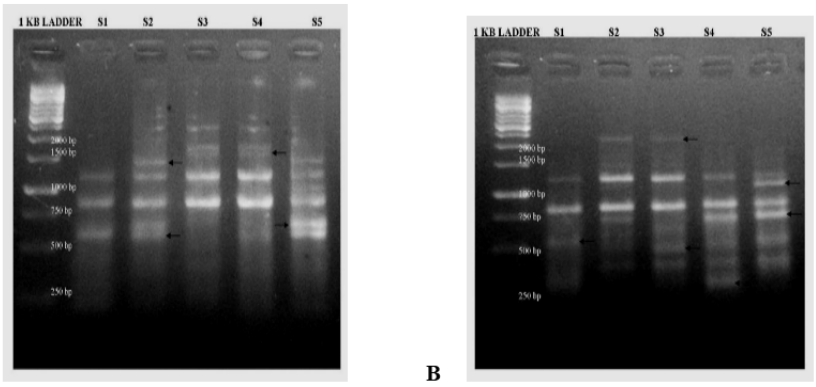

Fig 1: RAPD fingerprints *P. flavescens* for determination of polymorphism and taxonomic identities. The Primer OPC7 (A) and OPC10 (B) amplifies constant RAPD marker shared by the dragonfly, *P. flavescens*.

3.2 Population genetic analysis:

Genetic differentiation G_{st} by individual primers between populations was found to be 0.0478. Overall the calculated Nei's genetic diversity (h) was 0.4915 and Shannon's information index (I) was 0.6847 (Table2). The gene flow was calculated to be 9.9504. The dendrogram reveals the diversity among population from different locations: S1 populations are more diverse from the rest of the populations. S2 and S3 are less diverse to S4 and S5 (Figure2).

Table2: Distance matrix among 5 populations of *P. flavescens* showing Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Pop id	S1	S 2	S3	S4	S5
S1	******	0.8918	0.9195	0.8851	0.8851
S2	0.1145	******	0.9905	0.9830	0.9830
S3	0.0840	0.0095	******	0.9547	0.9547
S4	0.1220	0.0171	0.0463	******	10000
S5	0.1220	0.0171	0.0463	0.0000	*****

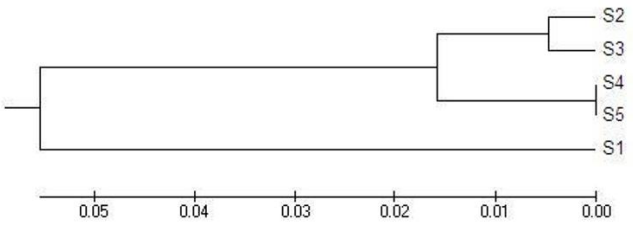

Fig 2: UPGMA dendrogram of genetic distance among 5 Indian populations of *P. flavescens* based on 5 RAPD primers.

4. Discussion

RAPD's are potentially powerful at many levels of identification from genus down to individual. A species with a broad distribution rarely has the same genetic makeup over its entire range. The relative allele frequency may differ dramatically from those at the opposite boundary. Distribution is one way that genetic variation can be preserved in large populations over wide physical ranges, as different forces will shift relative allele frequencies in different ways at either end.

Gene flow can act either to limit the ability of population to adapt to local conditions or to promote the spread of genes and genotypes throughout a species' range (Slatkin, 1987) [4]. Gene flow tends to reduce differences between populations over time. One reason for this to happen is migration.

Within the Indian subcontinent, several populations of globe skimmers arrive in Tamil Nadu after the north-east monsoon, while in the western region they arrive with the south-west monsoon (Nandini & Sane, 2009) [2]. Hence, *P. flavescens* is an obligatory seasonal migrant following the rain fronts of the inter-tropical convergence zone.

The analysis indicates that relationship between populations is irrespective of their geographical closeness. Some populations however showed greater gene flow. The Kerala and Mizoram populations which are geographically distant but share similar habitat showed more closeness. The other populations of geographically closer and similar habitats show greater gene flow.

Odonates are ancient life forms. It is remarkable that dragonflies have changed very little since the time of dinosaurs. They have survived by developing extreme efficiency in flight mechanism. Perhaps they will continue to preserve the species by evolving at a very slow rate. Moreover, the genetic fingerprinting technique serves to be efficient in characterizing the Odonates at species level.

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