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Assessment of genetic variation in *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) populations as revealed by mitochondrial cytochrome oxidase gene sequences

K. SRINIVASA MURTHY, R. RAJESHWARI, T. VENKATESAN and G. ASHOK KUMAR

National Bureau of Agriculturally Important Insects, Post Bag No. 2491, H. A. Farm Post, Bellary Road, Bangalore 560024, Karnataka, India.
E-mail: ksm239@rediffmail.com

ABSTRACT: The cytochrome oxidase gene (COI) was employed to assess the genetic variation in different populations of *Cotesia flavipes*. Partial sequences of the COI gene for the populations from Bangalore, Hyderabad, New Delhi and Shimla were analyzed to assess the homology and the usefulness of this genetic region for phylogenetic studies. PCR using COI-F and COI-R primers amplified a product of approximately 550 bp which was similar for all the four populations. Populations were by and large similar in the COI gene sequenced and there was no variation with our sequences and those of sequences worldwide. The multiple alignments were performed for four populations which revealed similarity of the partial COI gene sequences. Comparative analysis of partial sequences of COI gene produced a phylogenetic tree. Phylogenetic analysis revealed that all our populations were in a single clade with high boot strap value, suggesting highest similarity.

KEY WORDS: *Cotesia flavipes*, cytochrome oxidase, genetic variation

INTRODUCTION

The genus *Cotesia* is a large group of internal gregarious parasitoids of lepidopteran larvae. *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) is most widely used for biological control of several lepidopterous stem borers (Muirhead *et al*., 2006). The extent of parasitisation on *Chilo partellus* alone ranged from 2 to 50% in various states (Borah and Arya, 1995; Mohan *et al*., 1999). Inundative releases of the parasitoid resulted in decreased stem borer densities to the tune of 32–55% in various crops (Kfir *et al*., 2002).

Genetic divergence in geographically distinct populations has generally been interpreted and the existence of host-specific strains in *C. flavipes* has been postulated (Potting *et al*., 1997) to influence the host searching propensity and the parasitisation efficiency. Higher parasitisation in a location-specific strain which co–evolved with the host closely related to and ecologically similar to the target pest was observed (Hokkemen & Pimental, 1999). Therefore, intraspecific variability may result in a biologically aggressive population than others (Kankare *et al*., 2005) and influence the host-parasitoid dynamics in multi-species communities and tritrophic interactions. The knowledge of possible cryptic species is, therefore, essential for any ecological and evolutionary study.

Molecular studies of insects are becoming increasingly important in resolving genetic relationships. DNA–based approaches have been used to generate molecular markers that are useful for characterization of closely related or cryptic species for biological control (Dowton and Austin, 2001; Whitfield and Cameron, 1998). The most commonly analysed regions for resolving genetic relationships in closely related species and populations in Hymenoptera have been the 16S ribosomal DNA, 18S ribosomal DNA and the internal transcribed spacer 2(ITS2) of the nuclear ribosomal DNA and the mitochondrial genes, cytochrome oxidase I and COII (Hoy, 1994). Although these coding regions are generally conserved in composition and gene order, reliable sequence divergence at the species level and above has been indicated for many insect taxa (Shetter and Grinsell, 2003). The use of 16S gene was reported to assess the phylogeny of *Cotesia* sp. (Rattan *et al*., 2006).

Existing knowledge on the genetic diversity and phylogeography of the parasitoid is scattered. Investigations

were therefore carried out to study the variability in the populations of *C. flavipes* collected on maize crop from different agro-ecological regions. Using polymerase chain reaction (PCR), partial sequences of the COI for different populations were amplified to assess the homology or otherwise and the usefulness of this genetic region for phylogenetic studies is discussed.

MATERIAL AND METHODS

Stock culture and rearing of the parasitoid on *Chilo partellus* larvae

Nucleus cultures of the parasitoid populations were obtained from maize ecosystem in different locations of India, *viz*., Bangalore, Hyderabad, New Delhi and Shimla. Freshly emerged adults were transferred to plastic jars (1.0 litre capacity) fitted with brass mesh for ventilation. Cotton swab soaked in 50% honey was provided as food for the adults. Larvae of *Chilo partellus* reared on semi–synthetic diet as prescribed by Ballal *et al*. (1995) were utilized for parasitisation. Fourth-fifth instar larvae were offered to two–day–old adult parasitoid for 24 hours and the parasitized host larvae were transferred to vials (10 x 2.5 cm) containing artificial diet and reared till the formation of cocoons. The cocoons were then separated into another vial for adult emergence. The populations were maintained in the laboratory at an ambient temperature of 26 $\pm$ 1°C and 65% RH.

Isolation of DNA

Two adults of *C. flavipes* obtained from the larvae of *Ch. partellus* reared on semi-synthetic diet were frozen in liquid nitrogen and stored at –70°C until DNA extraction was carried out. Individual adults of each population were homogenized in a 1.5µl tube with 100µl of lysis buffer (200mM Tris HCl; pH 8.0, 70mM EDTA; 2M Sodium Chloride; 20mM Sodium metabisulphite) till a clear homogenate was obtained. About 35µl of 5% N Sodium lauryl Sarcosine was added and then incubated at 60°C for 2h. The mixture was centrifuged at 15,000 rpm for 15min and the supernatant was collected, 13.5µl of 10M ammonium acetate and 135µl of isopropanol were added and the tubes were left overnight at –20°C. The tubes were then centrifuged at 4°C for 15min at 15,000 rpm. The supernatant was discarded and the DNA pellet rinsed with 500µl of 70% ethanol and dissolved in 30µl of TE buffer. Genomic DNA isolated from individual wasps was stored at –20°C until use.

Cytochrome oxidase-I gene PCR

The PCR reaction was performed in 50 µl reaction volumes using Biorad icycler, 2µl DNA template, 5µl (10x) Taq assay buffer, 1µl dNTP's (each in 10mM concentration), 1µl forward and reverse primers (10 picomoles / µl), 0.25µl taq polymerase (1unit). The primers used to amplify the ITS-2 region were 5'-GGAGGATTTGGAAATTGATTAGTTCC-3' (forward) and 5'CCCGGTAAAATTAAAATATAAACTTC-3' (reverse). The thermal cycling condition for PCR consisted of 30 cycles (Den: 94°C for 1 min, Ann: 61°C for 1 min, Ext: 72°C for 2 min, with an initial denaturation: 95°C for 5 min and final extension at 72°C for 10 minutes). PCR products were electrophoresed on 1.8% agarose gel (ACROS) and visualized by ethidium bromide staining. Molecular standards were run along with the samples for reference.

Sequencing of PCR products

PCR products recovered from gel were extracted from the gel using the Qia Quick Gel Extraction Kit (Qiagen, Hilden, Germany). PCR products of the COI gene were sequenced directly with the corresponding PCR primers on both strands. The sequencing of the ITS-2 region was determined in ABI Prism 310 DNA sequencer using Big Dye Terminator Method. The partial sequences for the COI gene for Bangalore, Hyderabad, New Delhi and Shimla populations were aligned for phylogenetic analysis.

Phylogenetic analysis

Bioedit Version 7.0.4.1 program (Hall, 1999) was run to edit the sequences. The DNA sequences were aligned for the phylogenetic analyses using the CLUSTAL W programme. The evolutionary distances were computed by Kimura's two-parameter method (Kimura, 1980). The phylogenetic tree was constructed by the UPGMA method using MEGA version 3.1 (Kumar *et al*., 2004). The tree was evaluated using the bootstrap test (Felsenstein, 1985) based on 1,000 replicates.

RESULTS AND DISCUSSION

COI - PCR and sequencing

Cotesia flavipes populations from Bangalore, Hyderabad, New Delhi and Shimla were characterized using region-specific primers. An approximate size of 550 bp was amplified in PCR using COI-F and COI-R primers for *C. flavipes* showing intraspecific similarity between different populations (Fig 1). The partial sequence data was generated for COI gene to examine the genetic variation among populations of the *C. flavipes.* Sequences were confirmed as COI gene of mitochondrial DNA by Blastn similarity searching the Gen Bank database of National Centre for Biotechnology Information (Altschul *et al.,* 1997). DNA sequence variations in COI gene among populations of

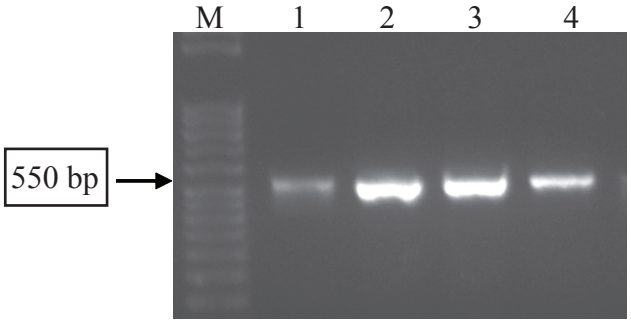

Fig. 1. Amplified PCR product of COI gene. Lane 1. 1 kb DNA ladder, *C. flavipes* from (lane 2, Bangalore; lane 3, Hyderabad; lane 4, Delhi; lane 5, Shimla)

C. flavipes were compared. The multiple alignments were performed for four populations which revealed similarity of the partial COI gene sequences.

Phylogenetic relationships within the populations of *C. flavipes* were analysed on the basis of sequence information of a fragment of mitochondrial COI gene which has a different mode of evolution and transmission and consists of highly conserved variable regions. Comparative analysis of partial sequences of COI gene produced a phylogenetic tree. Phylogenetic analysis revealed a single cluster with high boot strap value, suggesting similarity. Based on the dendrogram of the aligned partial COI gene sequences of our populations and from the GenBank sequences, the UPGMA based cluster can be divided in to two clades comprising of *C. flavipes* from Brazil (DQ232320), Kenya (DQ232317), Thailand (DQ232340), India (DQ232336), Pakistan (DQ232335), Jamaica (DQ232321), SriLanka (DQ232327), USA (DQ232330), New Guinea (DQ232316), Indonesia (DQ232337), Reunion (DQ232329), Mauritius (DQ232319), Japan (DQ232328), populations from New Delhi, Hyderabad, Shimla and Bangalore in the first clade while *C. flavipes* from India (DQ538818) in the second clade since the partial sequence of the COI gene has been submitted in the reverse order (Fig. 2). The partial sequence of COI gene of Bangalore population has been submitted to GenBank Accession number GQ853456.

Populations were by and large similar in the COI gene sequenced with no nucleotide divergence and there is considerable amount of variation between sequences and those of *C. flavipes* worldwide. Similar observations were made by Muirhead *et al*. (2006).

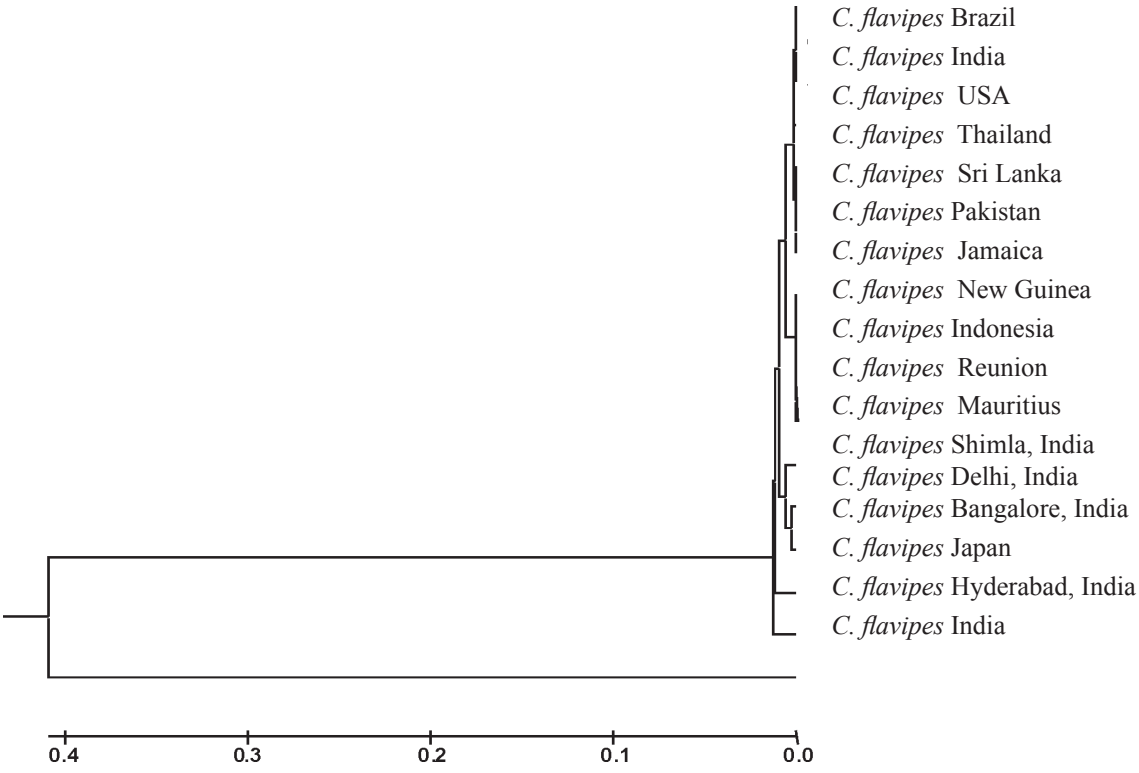

Fig. 2. UPGMA cluster analysis –based dendrogram depicting genetic relationships among *C. flavipes* populations The scale on top shows Jaccard's similarity values

Genetic differentiation is associated with host species rather than spatial isolation (Kankare *et al*., 2005). Host induced genetic variation as observed in thrips and other polyphagous insect pests such as *Helicoverpa armigera* (Scott *et al.*, 2003; Fakruddin *et al*., 2005) is rather unlikely to be a cause for such a variation as all the populations were obtained from a common host, *Chilo partellus*. However, variations in the genome level may be corroborated due to differences in the sequence of nucleotide insertion of host DNA as in the case of viral bodies. Similarly, the development of the parasitoid within the host body and the nutrition offered by the host probably influence variation. Spatial isolation naturally affects genetic structure within the species. The nature of the habitat, topography of the location and the predisposing ecological factors governing the population dynamics of the parasitoid in the area from where they were obtained appear to have greatly influenced the variations and seem to have offset the other rationale for the differences observed in the present studies. Differences in the biology of geographic populations have generally been interpreted as genetic divergence among strains, but evidence is lacking. Evaluation of the biological attributes of the various populations would ascertain this phenomenon.

In practice, better understanding of distribution of genetic variation at the intraspecific level would help identify superior populations for performance in the field as well as to evolve strategies for the establishment of effective strains for temperature and insecticide tolerance. Athough such empirical determination of genetic diversity can be obtained by evaluating morphological, physiological and biochemical traits, the information is scattered and not available for *C. flavipes.*

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