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Determination of Predator Prey Relationship in some Selected Coleopteran and Hymenopteran Species by DNA/PCR-based Molecular Analysis

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ABSTRACT

DNA/PCR based molecular gut analysis of *Coccinella septempunctata, Cheilomenes sexmaculata, Hippodamia convergens, Camponotus pennsylvanicus* and *Solenopsis invicta,* for six hemipteran pest species namely *Macrosiphum miscanthi, Aphis maidis, Schizaphis graminum, Bemisia tabaci, Empoasca kerri* and *Drosicha mangiferae* was done in the laboratory. Analysis was done by RAPD and Species specific primer techniques. A total of 175 fragments were amplified using 25 RAPD primers. 159 fragments were polymorphic, showing 91% polymorphism. The number of amplification products varied from 6 to 11, with an average of 7.00 per primer. With RAPD technique unique bands of some of the prey species were found in fed predators. A fragment of approx. 400 bp was observed with primer GLI-II in the DNA sample of *M. miscanthi* and fed *C. sexmaculata.* Another fragment of approximately 350 bp was observed with primer GLI-03 in the DNA sample of fed *H. convergens* and in DNA sample of *B. tabaci.* Species specific primer based gut analysis of *C. septempunctata, C. sexmaculata, H. convergens, C. pennsylvanicus* and *S. invicta,* for two major aphid pests namely; *S. graminum* and *A. maidis* was also done by using species specific mitochondrial cytochrome oxidase II primers derived from ribosomal RNA genes. Approximately 198 bp fragment of *A. maidis* with primer ClaCOIIF and ClaCOIIR1 was detected in *C. septempunctata, C. sexmaculata* and *H. convergens* specimens collected from wheat, brassica and fodder fields. Similarly approximately 111 bp fragment of *S. gramiinum* with primer GbCOIIF2 and GbCOIIR1 was detected in *C. septempunctata, C. sexmaculata* and *H. convergens* collected from wheat, brassica and fodder reconfirming their generalist predator trophic status. © 2012 Friends Science Publishers

Key Words: Coleoptera; Hymenoptera; Predator-prey relationship; DNA/PCR

INTRODUCTION

Coleopteran and hymenopteran species function in complex food webs as predators, prey of other predators and consumers of non-prey food. The role of generalist natural enemies in biological control and their interaction with prey species has received considerable attention (Oelbermann *et al*., 2008). Pests of agro-ecosystems are attacked by various natural enemies, which interact in complex ways (Sutherland *et al*., 2009). Coccinellids are the most studied group of insects. They are the major generalist predators of hemipterans such as aphids and scale insects, which are important pest species in agro-ecosystems (Batary *et al.,* 2007; Inayat *et al.,* 2011; Ruby *et al.,* 2011).

Feeding habits of ants are extremely varied. Many are carnivores and feed on flesh of animals, living or dead. Others feed on plants, fungi, sap, nectar and honeydew produced by sucking insects. A few ants are defoliators of forest trees (Ciesla, 2011).

The dietary breadth of coleopteran and hymenopteran predators still needs to be explored. The recent developments in molecular biology have made it possible to apply DNA based technologies for genomic gut analysis in a variety of animal species. Such studies help in expanding our understanding of trophic ecology of ecosystems (Weber & Lundgren, 2009). Among the several DNA based technologies, Random Amplified Polymorphic DNA (RAPD) gained importance due to its simplicity, efficiency and non-requirement of sequence information. RAPD markers have been used for the generation of genetic similarities and phylogenetic analysis of organisms (Krzywinski & Basanki, 2003). Different kinds of molecular markers can reveal different levels of genetic variation, making population genetic studies possible on a wide range

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of geographical scales. Properly performed, RAPD analysis is a useful and reliable tool for studying the ecology and genetic structuring in populations of many species (Pearson *et al.,* 2002). RAPD technique has also been used for detection of prey in the gut of predators (Zhang, 2007).

Gut content analysis allow prey consumption to be assessed after the feeding event (or events) occurred naturally in the field. Over the past decade species specific primers based molecular studies have been applied successfully to detect DNA of target prey within the guts of insects. Focusing observation efforts on a target prey can identify major predator groups that consume this species and thus help in the evaluation of the dietary breath of the predator species. Species specific primer-based studies have been helpful in tracking trophic interactions in food webs in ecosystems. It is a powerful, rapid, accurate and sensitive method for the detection of target prey in a predator (King *et al.,* 2008).

PCR results are affected by the species of predator (King *et al.,* 2008) and the time since the prey was ingested by the predator (Von Berg, 2008). The longer the duration of ingestion time, the lesser are the chances of prey DNA amplification and detection. The prey DNA detection of a single species is not affected by the sex of a predator, or the presence of other prey species in the gut contents (Hosseini *et al.,* 2008).

Interactions between natural enemies can be additive, synergistic or detrimental to biological control; careful assessment of the feeding ecology of generalist predators is required before they can be implicated for pest management. The present study was conducted to ascertain the dietary breath of the generalist predator species. The results will provide baseline information for further studies which will help in their use as biological control agents in agro-ecosystems.

MATERIALS AND METHODS

Six coleopteran, hymenopteran predators and six hemipteran prey species abundant in the field samples were selected for molecular gut analysis. Predator species comprised of *C. septempunctata, C. sexmaculata, H. convergens, C. pennsylvanicus, S. invicta* and *Formica rufa,* while prey species comprised of *A. maidis, M. miscanthi, S. graminum, B. tabaci, E. kerri* and *D. mangiferae.*

DNA extraction: The field collected specimens were immediately stored in 100% ethanol separately in 5 mL glass vials and genomic DNA was extracted using CTAB buffer method (Clark, 1997; Hamza *et al*., 2011). For each predator species, DNA extraction was made in three groups. These were the adult body of the individual (after feeding), whole larvae if present (in case of coccinellids) and only the head of the adult individual (used as control) in order to avoid contamination of DNA from other body parts. The whole body of each prey species was taken separately for DNA extraction.

Table I: RAPD primers with sequence, total number of bands (TNB), number of polymorphic bands (NBP), and mean band frequency (MBF) applied on insects

Primer Code	Sequence	TNB	NPB	MBF
GL DecamerA-01	CAGGCCCTTC	8	6	0.556
GL DecamerA-03	AGTCAGCCAC	7	6	0.805
GL DecamerA-05	AGGGGTCTTG	8	8	0.386
GL DecamerA-06	GGTCCCTGAC	6	8	0.349
GL DecamerA-07	GAAACGGGTG	7	5	0.492
GL DecamerA-15	TTCCGAACCC	6	6	0.318
GL DecamerA-17	GACCGCTTGT	7	7	0.435
GL DecamerA-18	AGGTGACCGT	8	5	0.345
GL DecamerB-07	GGTGACGCAG	8	8	0.466
GL DecamerB-10	CTGCTGGGAC	10	10	0.455
GL DecamerB-11	GTAGACCCGT	8	6	0.512
GL DecamerB-14	TCCGCTCTGG	7	5	0.389
GL DecamerC-02	GTGAGGCGTC	7	7	0.531
GL DecamerC-03	GGGGGTCTTT	6	6	0.363
GL DecamerC-11	AAAGCTGCGG	6	5	0.318
GL DecamerC-15	GACGGATCAG	8	8	0.466
GL DecamerC-19	GTTGCCAGCC	5	4	0.546
GL DecamerC-20	ACTTCGCCAC	6	6	0.515
GL DecamerD-11	AGCGCCATTG	10	9	0.472
GL DecamerI-03	CAGAAGCCCA	7	5	0.502
GL DecamerI-06	AAGGCGGCAG	8	7	0.420
GL DecamerI-11	ACATGCCGTG	7	7	0.454
GL DecamerI-12	AGAGGGCACA	8	7	0.533
GL DecamerJ-02	CCCGTTGGGA	7	6	0.455
GL DecamerJ-04	CCGAACACGG	8	8	0.461
GL DecamerJ-06	TCGTTCCGCA	7	7	0.523
GL DecamerJ-08	CATACCGTGG	7	7	0.384
GL DecamerJ-09	TGAGCCTCAC	6	5	0.459
GL DecamerJ-20	AAGCGGCCTC	8	8	0.398
GL DecamerK-02	GTCTCCGCAA	8	8	0.386
GL DecamerK-04	CCGCCCAAAC	6	6	0.323
GL DecamerK-08	GAACACTGGG	9	8	0.505
GL DecamerK-18	CCTAGTCGAG	8	8	0.466
GL DecamerK-19	CACAGGCGGA	7	7	0.532
GL DecamerK-20	GTGTCGCGAG	7	7	0.372
GL DecamerY-03	ACAGCCTGCT	9	7	0.455
GL DecamerY-05	GGCTGCGACA	8	8	0.392
GL DecamerY-07	AGAGCCGTCA	7	7	0.589
GL DecamerY-09	AGCAGCGCAC	7	6	0.567
GL DecamerY-10	CAAACGTGGG	8	8	0.421
GL DecamerZ-02	CCTACGGGGA	6	5	0.561
GL DecamerZ-04	AGGCTGTGCT	9	8	0.501
GL DecamerZ-06	GTGCCGTTCA	6	6	0.342
GL DecamerZ-08	GGGTGGGTAA	6	5	0.360
GL DecamerZ-10	CCGACAAACC	8	6	0.588

Table II: Accession number, primer sequence and amplified fragment size of species specific primers of different aphid species (Chen *et al.,* 2000)

Accession	Primer sequence	Species	Fragment size
ClaCOIIF ClaCOIIR1	CCAATTCTAACAATTAAAATTTTT GGA GAATAACATCATCTGATGAAATT AAA	*A. maidis*	198
GbCOIIF2 GbCOIIR1	GATGTTATTCACTCATGAACA GTCCAAAATATATTCCTGGG	*S. graminum*	111

Specimens were finely ground in 500 µL CTAB buffer and 5 µL of 20 µg/µL of proteinase-K added to each sample. After vortexing the samples were incubated at 65°C for 1 h (vortexing at 20 min interval). Samples were cooled

to room temperature. A 7 µL of 50 µg/µL RNase was added and incubated at 37°C for 2.5 h (vortexing at 30 min interval). Now the samples were centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant was transferred to fresh Eppendorf tube. 500 µL of Chloroform: isoamyl alcohol (24:1) was added. The mixture was vortexed and centrifuged at 10000 rpm for 10 min at room temperature. The upper aqueous layer was transferred to fresh Eppendorf tube. DNA was precipitated with 500 µL of 100% ethanol (-20°C). The supernatant was removed. The DNA pellet was washed with 70% ethanol. The precipitated DNA was centrifuged, air dried and re-suspended in 50µl sterile water ($3dH_20$). Total genomic DNA concentration was measured by spectrophotometer (AARI, USA) at 260nm wavelength. The quality of DNA was checked by running 5 µL of extracted DNA on 0.8% agarose gel prepared in 0.5X TBE buffer. The DNA samples giving smear in the gel were rejected as they were contaminated.

RAPD PCR analysis: Random Amplified Polymorphic DNA analyses were done using random decamer primers synthesized by Genelink Company, USA. Total of 43 RAPD primers (Table I) of seven different series (A, B, C, D, I, J & K) were used to amplify the genomic DNA of eleven insect species. The RAPD-PCR reaction was made by using 10x PCR buffer with $(NH_4)_2SO_4$, $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTp), decamer oligonucleotide primer and *Tag* DNA. RAPD-PCR was optimized with 2.5 µL (10X buffer), 3 µL of (3 mM $MgCl_2$), 5 µL dNTPs (25 mM for each), 2.5 µL of (0.01 mM gelatin), 2 µL RAPD-primer (15 ng), 0.2 µL *Taq* (1 unit) and 2.5 µL of 10 ng genomic DNA. The thermal cycler was programmed for 5 min initial denaturizing at 95°C, followed by 40 cycles comprising 1 min denaturizing at 95°C, 1 min primer annealing at 37°C and final extension at 72°C for 10 min. The amplified RAPD fragments were resolved by electrophoresis at 80 volts in 1.5% agarose gel with 0.5X Tris boric acid (TBE buffer) for two hours and were observed after ethidium bromide staining along with 1 Kb ladder. The DNA fragments were observed under UV trans-illuminator at 254-300 nm and photographed by using a Syngene still video system. Polymorphic bands and their occurrence frequencies were calculated. Also the mean band frequency was calculated (Table I).

Gut content analysis by species-specific primers: For gut content analysis, *C. sexmaculata, H. convergens*, and *C. septempunctata, C. pennsylvanicus* and *S. invicta* were captured live and brought to the laboratory from, wheat, brassica and fodder crop fields, and then killed by freezing. They were checked for the consumption of aphid *S. graminum* and *A. maidis* species. The protocol for species specific mitochondrial cytochrome oxidase II primers for major aphid species was used as given in Chen *et al.* (2000) with few modifications. PCR reactions, using aphid primers (Table II) were performed; 100bp ladder was used, PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide, and photographed under UV light.

RESULTS

Optimization and reproducibility of RAPD: Factors such as sequence of a primer, template quality and quantity, the type of thermal cycler and polymerase employed can influence the reproducibility of RAPD, but a standardized RAPD protocol can ensure the reproducible RAPD pattern (Khan *et al.,* 2005). Each PCR reaction was repeated 2-3 times for confirmation of its reproducibility. Reagent concentration was kept constant throughout the experiment. Three different concentrations of DNA (10, 15 & 20 ng/µL) were tested and 10 ng/µL DNA was found to be optimum. In the same manner 3 mM concentrations of $MgCl_2$ and 1 unit Taq was found to be optimum for amplification of RAPD fragments. Higher concentration of Taq and $MgCl_2$ caused smearing and lower concentrations produced lighter bands (Khan *et al.,* 2005).

Genetic characterization based on RAPD analysis: For genetic characterization of predator and prey species 43 RAPD primers were used. Out of these, 25 primers (Table I) produced polymorphic amplification, the remaining 18 produced monomorphic banding pattern and thus were excluded from the study. 175 fragments were amplified, and 159 were polymorphic. This showed 91% polymorphism which seems slightly higher than that obtained by Gadelhak and Enan (2005), who studied order Coleoptera and found 64% polymorphism by using 20 random primers. The number of amplification products produced varied between 6 to11 with an average of 7 per primer. The primer GLC-15 and GLK-08 amplified the maximum number of polymorphic bands (11); six primers amplified 10 bands whereas primer GLK-02 amplified 9 bands (Table I). Primer GLC-19 produced the minimum number of bands (5). The overall mean band frequency ranged between 0.323-0.805 with an average value of 0.673.

Predator-prey Relationship by Molecular Analysis of Gut RAPD analysis: The RAPD screening was broad enough to find the unique bands of some of the prey species in fed predators. A unique fragment of approximately 400 bp was observed with primer GLI-II in the DNA sample of *M. miscanthi* and fed *C. sexmaculata,* which was absent in *C. sexmaculata* used as control (Fig. 2). Another fragment of approximately 350 bp was found with primer GLI-03 in the DNA sample of fed *H. convergens*. Same fragment was found in the DNA sample of *B. tabaci* and it was absent in the *H. convergens* used as a control (Fig. 3).

Species-specific primers: This technology is superior to other molecular techniques such as monoclonal antibodies, due to the fact that it is quicker, less expensive and gives more defined results. PCR based gut content analysis is an established strategy but there are few records of its local use in arthropod predator gut content analysis.

By using species specific primers, it was possible to detect the specifically selected aphid species in the gut of coccinellid predators. A fragment of approximately 200 bp was found with primer ClaCOIIF and ClaCOIIR1 of

Fig. 1: Polymorphic RAPD banding pattern of eleven insect species; L: 1kb ladder; Lane 1: *C. septempunctata,* Lane 2: *C. sexmaculata,* Lane 3: *M. miscanthi.* Lane 4: *A. maidis.* Lane 5: *D. mangiferae,* Lane 6*: B. tabaci,* Lane 7: *E. kerri,* Lane 8: *H. convergens,* Lane 9*: C. pennsylvanicus,* Lane 10*: S. invicta,* Lane 11: *Formica rufa*

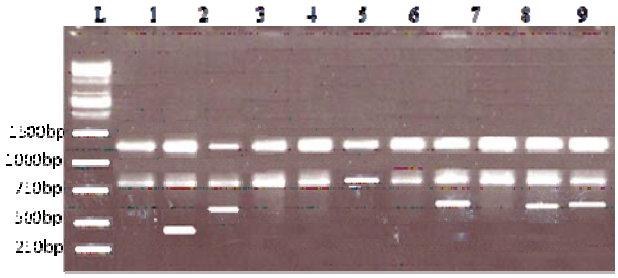


Fig. 2: Polymorphic RAPD banding pattern of *C. sexmaculata* with five prey species showing unique band of *M. miscanthi* in fed *C. sexmaculata* ;L: 1kb ladder; Lane 1: *C sexmaculata* (unfed) Lane 2: *C sexmaculata* (fed), Lane 3: *B. tabaci*, Lane 4: *A. maidis,*Lane 5: *D. mangiferae* , Lane 6: *E. kerri* Lane 7: *M. miscanthi*

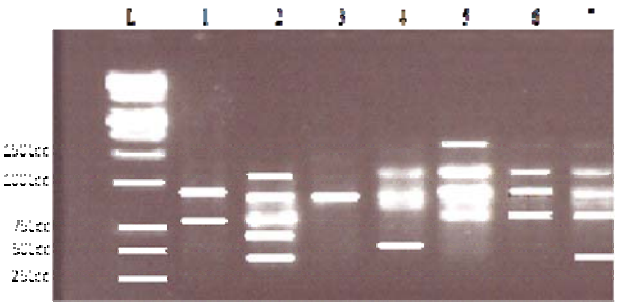
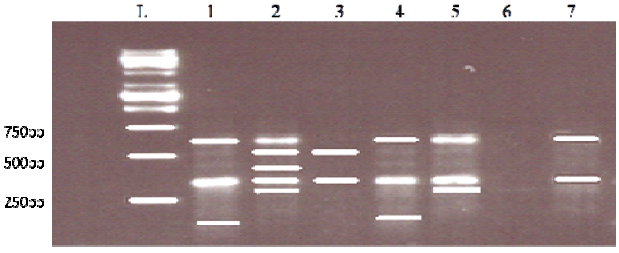


Fig. 3: Polymorphic RAPD banding pattern of *H.convergens* with five prey species showing unique band of *B.tabaci* in fed *H.convergens;* L: 1kb ladder; Lane1*: H. convergens* (unfed), Lane 2: *H. convergens* (fed), Lane 3: *M. miscanthi,* Lane 4: *E. kerri* ,Lane 5: *B. tabaci,* Lane 6: *D. mangiferae,* Lane 7: *A. maidis*



A. maidis in *C. septempunctata, C. sexmaculata* and *H. convergens* specimens collected from wheat and brassica (Fig. 4) and fodder fields (Fig. 5). Similarly a fragment of approximately 111 bp was found with primer GbCOIIF2 and GbCOIIR1of *S. graminum* in *C. septempunctata* and *C.*

Fig. 4: Amplification products *C. septempunctata, C.sexmaculata and H. convergens* collected from wheat and brassica crops using corn aphid; *A. maidis* primers on a 1.5% agarose gel. L is 100 bp DNA ladder Lane1*: C. septempunctat* from wheat, Lane 2 *C. septempunctata* from brassica, Lane 3: *C. sexmaculata* from wheat, Lane 4: *C. sexmaculata* from brassica, Lane 5: *H. convergens* from wheat Lane 6 *H. convergens* from brassica

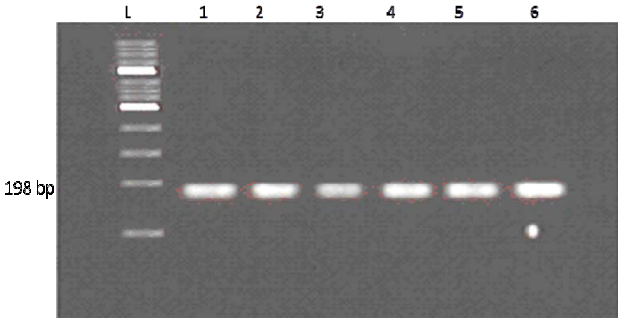
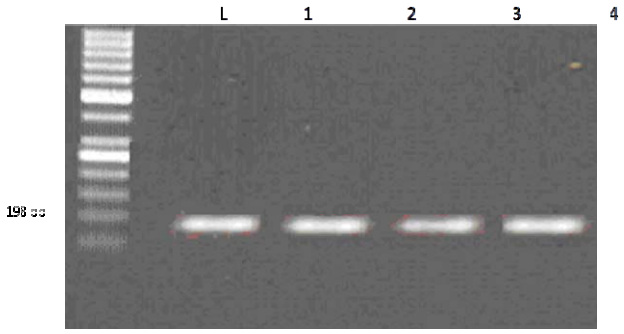


Fig. 5: Amplification products *C. septempunctata, C.sexmaculata and H. convergens* collected from fodder using corn aphid; *A, maidis* primers on a 1.5% agarose gel. L is 100 bp DNA ladder Lane1: *C. septempunctata,* Lane 2: *C. sexmaculata* , Lane 3: *H. convergens* and lane 4: *A. maidis*



sexmaculata and *H. convergens* collected from fodder (Fig. 6) wheat and brassica fields (Fig. 7). No prey DNA fragment was detected in the hymenopteran species.

DISCUSSION

The ability to identify prey remains within arthropod predators is essential for understanding the trophic inter-actions within complex food webs (Schmidt *et al*., 2009). Identifying prey remains within the guts of insect predators gives insight into the complex trophic interactions between predator communities and their prey. Observations of predation, manipulative field studies and feeding trials in the laboratory provide information on the feeding behavior of predators but may not account for the many interactions that occur between arthropods in the field. Although gut-content analyses have difficulties associated with their

Fig. 6: Amplification products *C. septempunctata, C.sexmaculata and H. convergens* collected from fodder using wheat aphid; *S. graminum* primers on a 1.5% agarose gel. L is 100 bp DNA ladder Lane1*: C. septempunctata,* Lane 2*: C. sexmaculata* , Lane 3*: H. convergens* , Lane 4*: S. graminum*

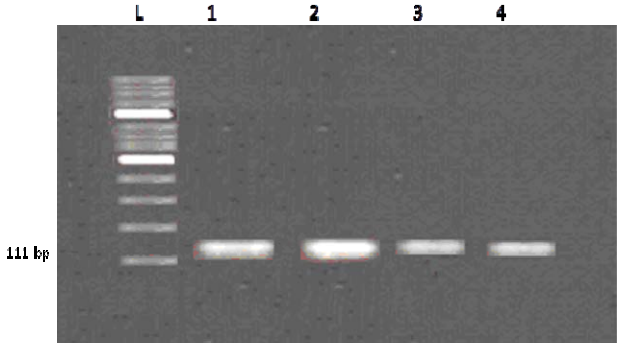
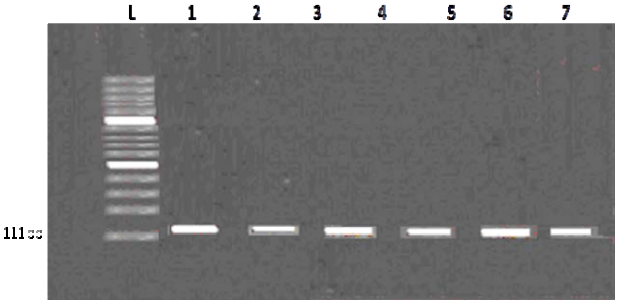


Fig. 7: Amplification products *C. septempunctata, C.sexmaculata and H. convergens* collected from wheat and brassica crops using wheat aphid; *S. graminum* primers on a 1.5% agarose gel. L is 100 bp DNA ladder Lane1*: C. septempunctata* from wheat, Lane2*: C. septempunctata* from brassica, Lane 3*: C. sexmaculata* from wheat, Lane 4*: C. sexmaculata* from brassica, Lane 5*: H. convergens* from wheat Lane 6 *H. convergens* from brassica



interpretation, quantification and potential sources of error, they do represent an extremely accurate means by which the feeding behavior of generalist predators can be assessed (King *et al.,* 2008). Gut-content analysis provides a valuable insight into the feeding ecology and trophic interactions between aphidophagous predators (Hardwood & Obrycki, 2005).

Gut content analysis of the insect predator species by RAPD PCR molecular technique showed presence of a similar unique DNA fragment in fed *H. convergens* predator and *B. tabaci* prey species. This fragment was absent in *H. convergens* used as a control. Likewise, a unique fragment was present in DNA sample of fed *C. sexmaculata*. Presence of similar fragment in *M. miscanthi* and its absence in *C. sexmaculata* (control) indicated existence of some trophic link between the predator and prey species. Such unique DNA fragments can be cloned and the predator prey relationship can be confirmed by development of Sequence-Characterized Amplified Region (SCAR) as was done by Zhang (2007). He studied predator prey relationship between *Propylaea japonica* (*Coleoptera: Coccinellidae*) and *B. tabaci.* A species-specific DNA fragment, 350 bp, was identified by random amplified polymorphic DNA analysis. This fragment was absent in other closely related or co-occurring prey species, cotton, and other select predator species. After cloning and sequencing the fragment, one pair of sequence-characterized amplified region (SCAR) primers was developed, which amplified a single band of 240 bp. Specificity tests performed with the primers showed the presence of the 240-bp band for *B. tabaci* in all developmental stages and both sexes, in adult *P. japonica* (Thunberg) fed on B. tabaci nymphs in the laboratory, and collected in cotton fields.

Single-plex species specific primer analysis confirmed the presence *S. graminum* and *A. maidis* prey species in the gut of *C. septempunctata, C. sexmaculata* and *H. convergens*. The presence of prey within the predators gut indicates that the target has been consumed (Hardwood & Obrycki, 2005). Absence of prey DNA fragments in the hymenopteran species may be because they are omnivorous and do not prefer aphid species rather they have been found to tend the aphids (Ho Jung *et al*., 2011).

The present work is the pioneer work in the area which has provided some baseline information about feeding habits of few generalist predators. This work can be expanded and dietary breadth of these and other insect predator species can be further carried out. For future studies, multiplex PCR assay can be performed in the area, by which DNA remains from several prey types can be amplified simultaneously (Harper *et al*., 2005). Apart from tracking predation of both pest and alternative prey in the same run, primers can be used that reveal intra-guild predation (Kuusk, 2009). In general, I believe that DNA-based gut-content analysis is be a valuable complementary tool future agro-ecosystem management projects of the area because it helps in generating knowledge about the dietary breadth of the insect predator species that would have been difficult with "non molecular" approaches.

In conclusion, gut-content analysis can provide a valuable insight into the feeding ecology and trophic interactions between aphidophagous predators and their prey. The RAPD screening showed unique bands of some of the prey species in field collected predators. Such fragments could be used for the development of SCAR marker for identification of species. Species specific primer based studies confirmed *C. septempunctata, C. sexmaculata, H. convergens* to be generalist predators.

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