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GENETIC ANALYSIS OF GREEN GARDEN LOOPER, *Chrysodeixis eriosoma* (LEPIDOPTERA: NOCTUIDAE: PLUSIINAE) USING MITOCHONDRIAL COI GENE SEQUENCES FROM INDIA

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ABSTRACT

Green garden looper, *Chrysodeixis eriosoma*, has emerged as one of the major insect pests of agronomic and vegetable crops causing considerable crop losses. In the present study, samples from eleven different localities of India were used for the investigation of genetic diversity of *C. eriosoma* by employing a fragment in the mtDNA gene-encoding cytochrome oxidase I (COI). Based on the partial COI gene, less nucleotide diversity ($\pi = 0.00314$) among Indian and global *C. eriosoma* populations were observed, whereas nucleotide diversity within Indian populations is nonsignificant ($\pi = 0.00079$). Additionally, phylogenetic analysis of COI sequences grouped all the Indian and global populations into one cluster. This is the first attempt for diversity assessment of *C. eriosoma* populations from India.

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1 Introduction

Insects are a diverse group of organisms and can use a wide range of plant species as hosts (Despres et al., 2007). Noctuidae is the second largest family in Noctuoidea, with about 1,089 genera and 11,772 species (Zhang, 2011). Plusiinae is the type subfamily of Noctuidae, has a cosmopolitan range distribution and comprises 500 species in four tribes and 36 genera worldwide (Ronkay et al. 2008). Morphologically Plusiinae are dark-colored moths having a similar general appearance with brighter silver gamma mark/golden patch on their forewing. It includes approximately 59 described species (Shashank & Longjam, 2014) including many widely distributed pests for various agronomic and vegetable crops of India (Twinkle et al., 2018). *Chrysodeixis eriosoma* (Doubleday, 1843), a member of this subfamily, commonly known as green garden looper is a worldwide serious polyphagous pest. It found throughout Asia, Europe, North, and South America and Oceania. It is also recorded from Australia, Fiji, New Zealand, Papua New Guinea and Tonga (Ronkay et al., 2010). In India, it is reported from Assam, Delhi, Maharastra, Tamil Nadu, Uttar Pradesh, Uttarakhand, Arunachal Pradesh, Himachal Pradesh, Kerala, Rajasthan and Meghalaya (Shashank & Singh, 2014). The green garden looper, *C. eriosoma* (Lepidoptera: Noctuidae), is one of the major constraints hampering productivity in crops globally. The larva of this species are predominantly feeding on the plants of families Solanaceae (Broza & Sneh, 1994; EPPO, 2004), Asteraceae (Compositae), Brassicaceae (Hill, 1983; Anonymous, 1984), Cucurbitaceae, Fabaceae, Poaceae including economical crops like tomato, beans, pumpkin, capsicum, tobacco (Martin & Workman 1986; Tripathi & Shari, 1992). The early instar larvae of this species feeds by rolling of leaf and webbing (CABI, 2007), while later instars voracious feeders and feed entire leaf leaving midrib and other large veins (Taylor & Kunjecku, 1983). The pest species was recorded first time from a pea plant in Delhi by Prasad et al., 1983. Further, in India, Pea, Soybean, Cabbage, Potato, Berseem, Cauliflower, Chickpea, Sunflower and Lentils are known to be most preferred hosts of *C. eriosoma* (Prasad et al., 1983; Saha & Saharia, 1983; Butani & Jotwani, 1984; Tripathi & Akhtar, 1986; Tripathi & Tripathi, 1988; Tripathi & Akhtar, 1988; Men et al., 1995; Sharma et al., 2008; Nair et al., 2017). Fecundity table and intrinsic rate of natural increase in *C. eriosoma* (Doubleday) was studied by Tripathi & Shari 1992. The genus *Chrysodeixis* comprises five species which were found in India, viz. *C. eriosoma*, *C. chalcites* (Esper, 1789), *C. acuta* (Walker, 1858), *C. minutus* (Dufay, 1970) and *C. permissa* (Walker, 1858). However, *C. eriosoma*, *C. chalcites*, *C. acuta* considered as sibling species and all are considered as a serious crop pest. These pest species usually identified by its morphological and genitalia variation within species but always confusing because of identical structures of genitalia with very little variation. So, proper identification of these species by various molecular analysis tools is very important for proper pest management. The aim of this study is to describe the basic information for molecular variability and population genetic

structure of *C. eriosoma* using mitochondrial marker cytochrome C oxidase (COI). This study particularly focused upon the DNA barcode gene, which has been found to be a promising method and is being used to identify species, subspecies, cryptic species of insect pest.

2 Materials and Methods

2.1 Sampling

Moth specimens were collected from eight different states i.e. Arunachal Pradesh, Delhi, Kerala, Himachal Pradesh, Uttarakhand, Rajasthan, Punjab, and Meghalaya and eleven different localities of India. The light traps method was used for the collection of adult moths. Further, collected specimens were processed by pinning, spreading, proper labeling, and preparation of wings and genitalia slides. All the specimens are preserved in NPC-IARI, New Delhi.

2.2 Morphology

Male and female specimens were identified morphologically by using a stereomicroscope. Genital characters are predominantly used for discriminating species of *C. eriosoma* and afford better diagnostic value than external wing patterns or coloration. More than 20 specimens were dissected to examine cases where there was a conflict between morphology-based identification and the DNA barcode results.

2.3 DNA extraction, COI amplification, and sequencing

The DNA was extracted from the individual of *C. eriosoma* using DNeasy Blood and Tissue Kit (Qiagen GmbH, Germany) following the manufacture's protocol. The forward LCO-5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3', and reverse HCO-5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' primers specific to COI were applied in this study (Folmer et al. 1994). PCR amplification was performed on a BioRad thermal cycler with the following conditions: initial denaturation for 5min at 94°C followed by 35 cycles of denaturing for 30s at 94°C, annealing for 40s at 47°C and an extension time of 40s at 72°C, with a final extension for 5min at 72°C. The optimized PCR conditions (per 25 µL) using 0.1 µL Dream Taq DNA polymerase (5U/µL), 2.5 µL of 10X PCR buffer with 2 µL of 25 mM MgCl₂, 0.5 µL of 10 mM dNTPs, 0.5 µL each of forward and reverse primers, 16 µL of nuclease-free water. Each PCR reaction mixture contained 2.0 ml of DNA template. The resultant PCR products were identified and sized by electrophoresis in a 1% TAE-agarose gel with 100 bp DNA ladder. Bands were visualized by ethidium bromide staining and samples yielded fragments of the expected size of 700 bp. Single bands were purified using a QIAquick PCR purification kit (Qiagen GmbH, Germany). Purified PCR products were sequenced directly in both directions using an automated sequencer (ABI prism® 3730 XL DNA Analyzer; Applied Biosystems, USA) at Scigenomics Lab, Cochin,

India. Authenticity of COI sequences obtained from this study was cross-checked with reference sequences of database. The sequence details were analyzed carefully and submitted to NCBI for the GenBank Accessions number (Table 1).

2.4 Data analysis

Nucleotide sequences for the isolates were compared with data deposited previously in GenBank using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Clustal W was used for multiple sequence alignment. Relationships among samples were then visualized by constructing a tree using the Maximum-likelihood method with 1000 bootstrap replicates based on p-distances. Following the removal of duplicate identical sequences from single locations, the analysis included 37 nucleotide sequences.

Evolutionary analyses were performed in MEGA6 (Tamura et al., 2013). The COI sequences newly obtained in this study were submitted to GenBank under Accession Nos. MN036462, MK861930, MK861931, MN036477, MN036467, MK861928, MK861929, MN036475, MN036478, MK861924, MK861925, MK861926, MK861927, MN036505, MN036466, MN036519, MK000724, MK840846, MK840845, MK861922, MK861923 and MF140488. These were combined with sequence data downloaded from GenBank for other country populations. Two sequences of *Spodoptera exigua* was used as an out group in the analysis. A total of 37 sequences (22 from this study and 15 sequences from different countries were downloaded from NCBI database) were used in diversity analysis. DnaSP 5.10.01 (Librado & Rozas, 2009) was used to estimate a series of population-level parameters including pairwise nucleotide diversity π and haplotype diversity h . Neutrality tests like

Table 1 Details of the sample data used in the analyses of *C. eriosoma* population

countries	Accession no./BOLD sample Id.	Specific location	Latitude	Longitude
India				
Arunachal Pradesh	MN036462, MK861930, MK861931	Khonsa	26.995	95.497
Delhi	MN036477, MN036467, MK861928, MK861929	IARI Campus	28.632	77.165
Kerala	MN036475	Pandimotta	8.827	77.216
Himachal Pradesh	MN036478	Nauni	30.856	77.164
Himachal Pradesh	MK861924, MK861925	Katraian	32.097	77.135
Himachal Pradesh	MK861926, MK861927	Dalang	32.508	77.006
Uttarakhand	MN036505, MN036466	Dehradun	30.344	77.998
Rajasthan	MN036519, MK000724	Durgapura	25.495	78.557
Punjab	MK840846, MK840845	Patiala	30.356	76.453
Punjab	MK861922, MK861923	Ludhiana	30.902	75.808
Meghalaya	MF140488	Umiam	25.676	91.843
USA	KM870778.1	Hawaii	-	-
USA	CNCLEP00083727	Hawaii	-	-
Pakistan	HQ991371.1	Malamjabba	-	-
Pakistan	HQ991185.1	Malamjabba	-	-
Papua New Guinea	KP850853.1	Morobe	-	-
New Guinea	USNM ENT 00209666	Madang	-	-
Canada	KX860528.1	-	-	-
Malaysia	BIOUG21481-C10	-	-	-
French Polynesia	CCDB-34794-A10	Marquesas Islands	-	-
French Polynesia	USNM ENT 01068037	Marquesas Islands	-	-
Australia	ANIC.000788	Queensland	-	-
Australia	10ANIC.08304	Queensland	-	-
China	NHMO-DAR-10552 ARB00009625	Nantong	-	-
China		Yunnan	-	-
Seychelles	USNM ENT 01068096	Aldabra Atoll	-	-
Outgroups				
<i>spodoptera exigua</i>	ABOLA884-15	India	-	-
<i>spodoptera exigua</i>	BBLOC1615-11	India	-	-

Table 2 Genetic variability of COI sequences

	Rest of the world	India	Total
Sample size	15	22	37
No. of haplotypes (h)	4	2	6
Haplotype diversity (Hd)	0.638	0.091	0.704
Nucleotide diversity (π)	0.00348	0.00079	0.00314
No. of segregating sites (S)	11	5	15
Fu and Li's <i>F</i>	-2.66343	-3.06643	-3.62503 (**, $P < 0.02$)
Fu and Li's <i>D</i>	-2.53453	-2.91519	-3.63130 (**, $P < 0.02$)
Tajima's <i>D</i>	-1.74928	-1.98725	-1.92787 (*, $P < 0.05$)
Fu's <i>F</i>	1.103	1.274	-0.045

Fu and Li's *F* and *D* and Tajima's *D* values, significant ($P < 0.05$) values, while others have $P > 0.10$

Tajima's *D* (Tajima, 1989), Fu and Li's *D* (Fu & Li, 1993), and Fu's *FS* (Fu, 1997), were also calculated by DnaSP 5.10.01 (Librado & Rozas 2009) for investigating the historical population demographics and testing whether the sequences conformed to the expectations of neutrality.

3 Results

3.1 Genetic diversity of *C. eriosoma* based on COI sequence data

Twenty-two individuals of *C. eriosoma* were collected from eleven different localities of India. Other 15 sequences were downloaded from the NCBI database from nine countries based on the spread of *C. eriosoma* (Table 1). The sample size disparity is mainly based on the lack of sequence information from different countries. 37 sequences were used for analysis. The mean total nucleotide composition is A 29.8%, T 40.1%, C 15.5%, and G 14.6% in the sequences. The average AT content is 69.9% and the CG is 30.1%. This high AT content (69.9%) is a general feature of the COI region in arthropods and is comparable with other studies on insect and mite taxa. Furthermore, test statistics were generated for the 37 COI sequences in which the rest of the world group and India were analyzed as discrete units in the case of the diversity and selective neutrality tests (Table 2). Surprisingly, there is a lack of variation in COI sequences. A total four haplotypes in the rest of the world group and two haplotypes in India were observed. There is negligible nucleotide diversity (π) 0.00314 among the total world population of *C. eriosoma* (Table 2). Demographic history changes were analyzed for two groups through neutrality tests.

3.2 Phylogenetic analysis

The Maximum-likelihood tree (ML tree based on Kimura three-parameter distance at 2000 iterations) was constructed based on 37 sequences including 22 sequences from the present study and 15

sequences from GenBank and outliers (Table 1) using MEGA 6.0. Based on the strict consensus ML tree, all populations cluster together in a single group. The phylogenetic tree had a total branch length of 0.14211 base substitutions per site. The tree shows that there is no clustering associated with geographic origin. However, the samples from the different localities are grouped with Indian samples as a base. This suggests that there is a homogeneity in the *C. eriosoma* populations.

4 Discussion and conclusions

Results from the present study have confirmed that *C. eriosoma* populations across different countries are genetically homogeneous. However, based on *cox1* analysis, high genetic homogeneity among different populations is evident by less nucleotide variation. Furthermore, the lack of genetic variability was also represented by Maximum-likelihood tree (Figure 1). It has been reported that during the expansion of the population, many insect species exhibit less genetic variation as a result of bottleneck and founder effects (Lindholm et al., 2005; Hawley et al., 2006). Less genetically variable populations can be considered as only a subset of the genetic diversity present in the native population, and furthermore, if populations are small, there must be a decreased genetic diversity (Roderick, 2004). However, from the present inter- and intra-population study and data analysis of *C. eriosoma*, it can be concluded that no genetic divergence exists among Indian populations. Further, *C. eriosoma* from other countries belongs to a different ancestral history and are homologous to Indian populations. The homogeneity observed across the populations of *C. eriosoma* indicates its poor ability to overcome the management hurdles and also shows its recent spread across the country. This simplifies the designing of both traditional and biotechnological management strategies. This is the first attempt to generate molecular data for this emerging pest from different parts of India.

Figure 1 Maximum-likelihood (ML) phylogenetic consensus tree generated from the COI gene sequences of *C. eriosoma*. The consensus tree was inferred from 2000 bootstrap replicates.

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Disclosure statement

The authors declare that they have no competing interests.

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