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Molecular characterization of intraspecific variations in *Helicoverpa armigera* (Hübner) populations across India

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Abstract

Aim: The present study was undertaken to explore the genetic diversity among *Helicoverpa armigera* populations from varied geographic regions of India using mitochondrial cytochrome c oxidase I (COI) gene fragments.

Methodology: The larval specimens of *H. armigera* collected from 20 locations were subjected to DNA extraction, PCR amplification of target gene, sequencing and then multiple sequence alignments.

Results: Based on COI sequence data, high levels of genetic differentiation among some *H. armigera* populations were detected, but divergence existing was not high enough to delineate them as separate species. The Indian population as a whole exhibited similarity with global genetic assemblage. Significant negative neutrality test indices and unimodal mismatch distribution further supported that this insect experienced a demographic expansion in the past. The phylogenetic tree and median-joining haplotype network indicated that genetic similarity was not related with geographic proximity of populations.

Interpretation: Differences based on genetic analyses indicate considerable subspecific level variations among *H. armigera* populations of India. However, there is no existence of any unidentified cryptic species of *H. armigera* in the country.

Key words: COI gene, Diversity, Genetic structure, Haplotype, Phylogeny.

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Introduction

The Heliiothinae subfamily of noctuids includes some of the most damaging pests of agricultural importance throughout the world (Mitchell and Gopurenko, 2016). Among the different *Helicoverpa* or *Heliothis* species existing worldwide, three species that have been reported from India are *Helicoverpa armigera* (Hübner), *Helicoverpa assulta* (Guenée) and *Heliothis peltigera* (Denis and Schiffermüller), of which *H. armigera* is the most serious one (Ranjith and Chellappan, 2015). In India, it has been recorded feeding on 181 species of plants, distributed in nearly 45 families and out of these 96 species are agriculturally important crop plants (Srivastava et al., 2010). Depending on the crop, *H. armigera* induced damage can lead to 50 to 90 percent of yield loss (Chakravarty et al., 2018). The extent of damage caused by this insect on various crops under different agro-climatic zones has elevated its status to “a pest of national importance” in India (Sharma et al., 2010). The damage it causes is estimated to cost around 2 billion US dollars each year, apart from the environmental and socio-economic costs related to its control (Tay et al., 2013).

Looking at its ability to thrive on several hosts and its flexibility in rapidly developing resistance to almost all classes of insecticides, including *Bacillus thuringiensis*-derived toxins (Ranjith et al., 2010), it is doubted that there is an existence of strong genetic variability within Indian populations of *H. armigera*. The problem of resistance is estimated to be a few folds to few hundred folds to different groups of insecticides in various regions of our country (Armes et al., 1996). Even the resistance is also reported in *H. armigera* populations from those places in tribal regions where chemical control measures are hardly adopted (Deepa and Srivastava, 2011). On the other hand, sensitivity against various groups of insecticides also varies greatly among populations collected from different agro-climatic zones (Chaturvedi, 2013), arising the doubt regarding the existence of possible differences in the population genetic structure of Indian *H. armigera*. The different larval colour morphs of *H. armigera* also exhibit a differential susceptibility against various insecticides (Chakravarty et al., 2020), thereby rendering its management all the more difficult. There are also reports regarding Indian populations of *H. armigera* exhibiting differential responses to parasitoids (Romeis and Shanower, 1996) and pheromones (Tamhankar et al., 2003).

H. armigera is also a known facultative migrant (Nibouche et al., 1998). Its ability to undertake long-distance flights may cause intermingling of exotic populations with native ones, giving rise to the possibilities of developing new species and subspecies. Likewise, in diverse habitats existing such as those in India, the tendency to fly across different agro-climatic zones and mixing with native populations thriving on various host plants could lead to the possibility of occurrence of unknown subspecies of *H. armigera* (Behere et al., 2013). These possible changes in

the genetic make-up of the insect population due to the formation of new species or subspecies again could be the reason for differential susceptibility or resistance development to various selection pressures in different agro-climatic zones. Limited studies regarding the existence of phenotypic differences among varied host-associated populations of *H. armigera* (Gill et al., 2015) has further hinted that the populations occurring at different geographical locations and on different host plants across India are genetically diverse.

The adaptability of any species to environmental change largely depends on the genetic structure of its populations (Sambathkumar et al., 2017). Thus, understanding the genetic diversity in Indian *H. armigera* has become necessary to elucidate the actual cause for the occurrence of such variations in the physiological and behavioural characteristics of this pest in the country, as well as for development and deployment of long term effective management strategies (Subramanian and Mohankumar, 2006). Molecular markers are considered as important tools for assessment of inter-population genetic differences in insects (Sun et al., 2011). The cytochrome c oxidase subunit I (COI) region of mitochondrial DNA is particularly useful for determining intra- and inter specific complexities, as well as in elucidating phylogenetic relationships at the genus and species level, and within families (Hallerman, 2021). There are also several evidences that have proved that variations in the mitochondrial DNA genes influence the process of speciation in closely related cryptic species in several insect orders, including lepidoptera (Karthika et al., 2019). Hence in the present study, we sought to determine the variations within Indian populations of *H. armigera* based on COI gene.

Materials and Methods

Sample collection and DNA extraction: Larvae of *H. armigera* were collected from 20 different geographical locations falling under four major pulse growing zones of India, during March, 2015 to February, 2018. These locations were Ludhiana (HLUD; 30°54' N, 75°48' E), Hissar (HHIS; 29°10' N, 75°42' E), Delhi (HDHL; 28°64' N, 77°16' E), Pantnagar (HPNT; 29°01' N, 79°48' E) and Udaipur (HUDP; 24°34' N, 73°42' E) of North West Plain Zone; Kanpur (HKNP; 26°49' N, 80°27' E), Ayodhya (HFZB; 26°54' N, 81°82' E), Varanasi (HVNS; 25°15' N, 82°52' E), Samastipur (HSMP; 25°98' N, 85°67' E) and Cooch Behar (HCBR; 26°40' N, 89°38' E) of North East Plain Zone; Jabalpur (HJBP; 23°21' N, 79°96' E), Raipur (HRPR; 21°14' N, 81°42' E), Nagpur (HNGP; 21°08' N, 79°04' E), Akola (HAKL; 20°42' N, 77°01' E) and Pune (HPNE; 18°31' N, 73°51' E) of Central Zone, and Hyderabad (HHYD; 17°50' N, 78°27' E), Raichur (HRCR; 16°11' N, 77°19' E), Bagalkot (HBGK; 16°10' N, 75°41' E), Bengaluru (HBGL; 13°07' N, 77°56' E) and Coimbatore (HCBT; 11°01' N, 76°92' E) of South Zone. The species identity of collected specimens was confirmed as *H. armigera* morphologically using keys provided by Passoa (2014). The

samples were then stored in 95% ethanol at -20°C for performing the molecular investigations. The genomic DNA was extracted separately from four individual late instar larvae of each location (population). The extraction was carried out from the larval skin following the procedure described by Murray and Thompson (1980) with some modifications. The quality of extracted DNA samples was checked on 0.8% agarose gel and quantified using UV spectrophotometer (Ellico® SL191) as per Anno *et al.* (1998).

PCR amplification and sequencing of COI gene: The DNA extracts were subjected to PCR amplification of a 658 bp region of the 5' terminus of the COI gene following standard protocols as described by Shashank *et al.* (2014) using the primers LCO1490 (forward) and HCO2198 (reverse) as described by Folmer *et al.* (1994). The amplifications were performed in a 25 µl volume reaction mixture containing 2 µl of DNA template (100ng), 2 µl of PCR buffer (10X), 0.5 µl of Taq polymerase (1U, GeNei™), 1 µl each of specific primers (10pmol), dNTP (2mM) and MgCl₂ (2mM), and 17.5 µl of nuclease free water. Thermo cycling consisted of following steps: initial denaturation at 94°C for 5 min; followed by 30 cycles of amplification, each cycle with the following schedule: denaturation for 30 sec at 94°C, annealing for 45 seconds at 50°C and extension for 1 min at 72°C. This was followed by final extension at 72°C for 5 min. The amplification products were electrophoresed on 1% agarose gel having 0.05 µg ml⁻¹ ethidium bromide and sequenced by Sanger's method using ABI3730xl DNA sequencer (Applied Biosystems, USA) and Big Dye Terminator v3.1 kit (Applied Biosystems, USA) at M/s Eurofins Analytical Services India Pvt. Ltd. (Bengaluru, India).

Sequences alignment and data analysis: All the *H. armigera* COI gene sequences were imported into FASTA format for alignment and trimming in ClustalW algorithm by setting default parameters in the MEGA version 6.0 software package (Tamura *et al.*, 2013). Sequences were analyzed based on homologs of the gene when subjected to nucleotide BLAST (Basic Local Alignment Search Tool) of NCBI (<https://www.ncbi.nlm.nih.gov/>) and then submitted to NCBI for obtaining GenBank accessions. DnaSP version 5.0 software (Rozas *et al.*, 2003) was used to analyze the diversity indices like the number of haplotypes (Hn), haplotype diversity (Hd), nucleotide diversity (π), number of polymorphic sites (S) and average number of nucleotide differences among haplotypes (k). Further, to ascertain demographic history and evolutionary neutrality of *H. armigera* COI sequences, tests like Tajima's *D* (Tajima, 1989) and Fu's *F_s* (Fu, 1997), and mismatch distribution analysis (Rogers and Harpending, 1992) were also performed using DnaSP 5.0. The average pairwise sequence divergences among *H. armigera* populations were estimated using the Kimura 2-parameter distance model (Kimura, 1980) and displayed graphically in a neighbour-joining (NJ) tree, using MEGA 6.0, with a confidence level of 1000 bootstrap replicates. The number of sites with transitions (Ts) and transversions (Tv) from aligned sequences,

analysis of molecular variance (AMOVA) and pairwise genetic differentiation (F_{ST}) between populations were calculated in Arlequin version 3.5 (Excoffier and Lischer, 2010). The levels of genetic differentiation were categorized as $F_{ST} < 0.05$ (low), $0.05 \leq F_{ST} < 0.15$ (moderate), $0.15 \leq F_{ST} < 0.25$ (high), $F_{ST} \leq 0.25$ (very high). The haplotype network was constructed using a median-joining algorithm in Network 4.6.1.1 software (Bandelt *et al.*, 1999).

Results and Discussion

The sequences for 80 individuals sampled across India showed high similarity (100 percent query coverage and 99 percent identity) to *H. armigera* COI gene sequences already deposited in the NCBI-GenBank database, and thus the specimens were confirmed to be *H. armigera* and used for diversity analyses. Further, for comparison of Indian populations with global genetic assemblage, 20 additional COI gene sequences were mined from NCBI database. These sequences represented populations of 14 different countries outside India, based on the spread of *H. armigera*. Inclusion of these additional sequences produced a final alignment of 620 base pairs for 100 individuals. Sample size disparity is due to lack of sequence information from different countries. The sequence region showed high Adenine (A) + Thymine (T) content (30.35% A and 40.64% T, averaged across the multiple sequence alignment), in accordance with insect mitochondrial DNA (Sambathkumar *et al.*, 2017; Shashank *et al.*, 2018). The mean composition of Cytosine (C) and Guanine (G) in the sequences was 14.85% and 14.16%, respectively (Table 1).

The molecular diversity indices generated for the 100 COI gene sequences, in which Indian and foreign sequences were analyzed as discrete units, indicated about the existence of considerable differences among them (Table 1). A total of 52 polymorphic sites were observed in the COI gene sequences of the studied populations, showing both transitional and transversal substitutions. The nucleotide diversity (π) was found to be low in case of both the Indian (0.0910) as well as foreign (0.00269) populations. However, the populations were characterized by high values of haplotype diversity (Hd), i.e., 0.954, 0.853 and 0.948 for Indian, foreign and combined population sets, respectively. Furthermore, among the different Indian populations of *H. armigera*, the populations from North West Plain Zone had the highest number of haplotypes (Hn = 13) and gene diversity (Hd = 0.953±0.028), while the South Zone populations exhibited highest nucleotide diversity (π = 0.01410) and the average number of genetic differences among haplotypes (k = 8.742). In contrast, the Central Zone population set was lowest in all concerns (Hn = 9, Hd = 0.847, π = 0.00500 and k = 3.100).

The mismatch nucleotide frequency spectra (distribution of pairwise differences) for the studied pest species are shown in

Table 1 : Molecular diversity indices and demographic history parameters of different *H. armigera* populations, assessed using mitochondrial COI gene sequences

Index	Indian population					Foreign population	Total
	NWPZ	NEPZ	CZ	SZ	Pooled		
n	20	20	20	20	80	20	100
Hn	13	11	9	12	39	8	43
Hd ± SD	0.953±0.028	0.921±0.037	0.847±0.061	0.949±0.028	0.954±0.012	0.853±0.059	0.948±0.028
π	0.00750	0.00603	0.00500	0.01410	0.00910	0.00269	0.00805
k	4.647	3.737	3.100	8.742	5.642	1.663	4.980
S	16	19	14	36	48	6	51
Ts	10	10	8	9	19	6	22
Tv	8	10	7	27	32	0	32
<i>Nucleotide composition (Relative values)</i>							
A (%)	30.56	30.35	30.24	30.34	30.36	30.28	30.35
C (%)	14.94	14.79	14.85	14.83	14.85	14.89	14.85
G (%)	13.90	14.12	14.10	14.48	14.16	14.11	14.16
T (%)	40.60	40.74	40.81	40.35	40.63	40.72	40.64
<i>Neutrality tests</i>							
Fu's <i>F_s</i>	-4.1487*	-2.8853 ^{NS}	-1.6368 ^{NS}	-0.5794 ^{NS}	-22.8404**	-2.9480 ^{NS}	-25.4363**
Tajima's <i>D</i>	-0.3161 ^{NS}	-1.2810 ^{NS}	-0.9845 ^{NS}	-0.5487 ^{NS}	-1.3569*	-0.1542 ^{NS}	-1.5732*

Abbreviations - n: number of sequences, Hn: number of haplotypes, Hd: haplotype diversity, SD: standard deviation of haplotype diversity, π: nucleotide diversity, k: average number of nucleotide differences (genetic differences) among haplotypes, S: number of polymorphic (segregating) sites, Ts: number of sites with transitions, Tv: number of sites with transversions, A: Adenine, C: Cytosine, G: Guanine, T: Thymine, NWPZ: North West Plain Zone, NEPZ: North East Plain Zone, CZ: Central Zone, SZ: South Zone. Asterisks represent significance levels: * $P < 0.05$, ** $P < 0.01$; NS: Non-significant ($P > 0.10$). Here "Pooled" denotes all the populations occurring in four major pulse growing zones of India and "Total" denotes combined set of Indian and foreign populations.

Fig. 1. The observed distribution was unimodal for the Indian, foreign as well as combined population sets, indicating a demographic expansion in the past. This result was further supported by significant negative values in Tajima's *D* test (-1.3569 and -1.5732, $P < 0.05$) and Fu's *F* test (-22.8404 and -25.4363, $P < 0.01$) for the pooled Indian and total populations, respectively (Table 1). Significant and negative neutrality test indices also indicate an excess of low-frequency polymorphism and are in accordance with either purifying selections or population expansion. These results are also congruent with earlier studies on several lepidopteran pest population diversity analyses from India such as *Leucinodes orbonalis* (Karthika et al., 2019), *Tuta absoluta* (Shashank et al., 2018), *Maruca vitrata* (Chatterjee et al., 2019) and *Pectinophora gossypiella* (Naik et al., 2020), apart from *H. armigera* (Leite et al., 2014) in Brazil. The AMOVA analysis (Table 2) revealed that 93.51% of total genetic variation was partitioned within Indian and foreign population sets and the remaining 6.49% was partitioned among populations ($F_{ST} = 0.06494$, $P < 0.01$). Additionally, only 13.23% genetic variation was among the Indian populations from different zones, while 86.77% genetic variation was within zone populations ($F_{ST} = 0.13229$, $P < 0.001$). This amount of variance is highly significant and reveals that there is considerable population genetic structure.

The level of intraspecific differentiation among Indian populations was further assessed by calculating the pairwise Kimura-2-Parameter based genetic distance and genetic differentiation (F_{ST}) values (Table 3). Corrected pairwise genetic distances among studied populations varied from 0.000 to 0.040 with highest evident between the Coimbatore and Kanpur populations of South and North West Zones, respectively. The genetic distance for most of the specimens from geographically adjacent locations ranged from 0.000 to 0.005, indicating not much variation within the populations of these locations from each other. The intra-population divergence for this gene was also found to be negligible. However, some of the populations collected from distant locations also exhibited very high similarity. This reveals that isolation by distance is not always expected in species like *H. armigera* having high mobility (Behere et al., 2013). Overall mean genetic distance was 0.015. The pairwise F_{ST} values ranged from -0.076 to 0.649, and many of them were significantly different. Out of 190 comparisons, only 35 showed low to moderate differentiation and the remaining showed high to very high genetic differences. These results also marked out signs of significant spatial population divergence.

Topologies of neighbour-joining tree indicated that all the studied Indian populations belonged to a single major clade,

Fig. 1 : Frequencies of the observed and expected pairwise nucleotide differences (the mismatch distribution) in *H. armigera* (A: Indian populations; B: Foreign populations; C: Combined set of Indian and foreign populations). X-axis (x): number of pairwise differences; Y-axis (y): frequency of mismatches; Freq. Exp.: frequency expected (green dashed line) and Freq. Obs: frequency observed (red solid line).

Table 2 : Analysis of molecular variance (AMOVA) for the mitochondrial COI gene sequences among and within different populations of *H. armigera*

Source of variation	df	Sum of squares	Variance components	Percentage of variation
A. Comparison of Indian and foreign population sets				
Among populations	1	7.847	0.16913 Va	6.49
Within populations	98	238.663	2.43533 Vb	93.51
Total	99	246.510	2.60446	100
Fixation index, F_{ST} : 0.06494**				
B. Comparison of Indian population sets occurring in different zones				
Among zones	3	30.713	0.38546 Va	13.23
Within zones	76	192.150	2.52829 Vb	86.77
Total	79	222.863	2.91375	100
Fixation index, F_{ST} : 0.13229***				

Asterisks represent significance levels: ** $P < 0.01$, *** $P < 0.001$; df: Degree of freedom

however, distinct subclades were also observed (Fig. 2). But within these subclades, there were no significant genealogical branches or clusters of samples corresponding to sampling sites or zones. *H. armigera* COI gene sequences deposited in the

GenBank database from other countries also shared similarity with studied sequences, indicating that Indian populations are ancestrally related to the world population. However, isolates from Australia and New Zealand formed a distinctive separate

Table 3: Pairwise F_{ST} (below diagonal) and genetic distance (above diagonal) amongst different populations of *H. armigera* in India based on mitochondrial COI gene sequences

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
HLUD	-	0.011	0.006	0.008	0.010	0.011	0.008	0.008	0.010	0.016	0.011	0.011	0.010	0.011	0.011	0.011	0.011	0.010	0.021	0.031
HHIS	0.145	-	0.011	0.013	0.015	0.020	0.013	0.016	0.011	0.018	0.018	0.018	0.011	0.013	0.013	0.018	0.013	0.011	0.028	0.038
HDHL	0.215	0.098	-	0.015	0.003	0.015	0.002	0.005	0.003	0.013	0.006	0.006	0.010	0.015	0.005	0.016	0.005	0.006	0.020	0.030
HPNT	0.319	-0.019	0.302	-	0.016	0.016	0.016	0.016	0.018	0.025	0.020	0.020	0.015	0.020	0.016	0.020	0.020	0.015	0.023	0.033
HUDP	0.181	0.428	0.571	0.287	-	0.016	0.002	0.005	0.003	0.013	0.006	0.006	0.010	0.005	0.005	0.006	0.008	0.010	0.018	0.028
HKNP	0.145	0.207	0.402	0.127	0.280	-	0.016	0.016	0.018	0.025	0.020	0.020	0.018	0.020	0.020	0.020	0.016	0.018	0.030	0.040
HFZB	0.357	0.426	0.242	0.148	0.467	0.111	-	0.003	0.002	0.011	0.005	0.005	0.008	0.003	0.003	0.005	0.006	0.008	0.018	0.028
HVNS	0.152	0.183	0.204	0.256	0.467	0.098	0.067	-	0.005	0.015	0.006	0.006	0.011	0.006	0.006	0.006	0.010	0.011	0.020	0.030
HSMP	0.333	0.418	0.580	0.389	0.586	0.111	0.500	0.429	-	0.010	0.006	0.006	0.006	0.002	0.002	0.006	0.005	0.006	0.016	0.026
HCBR	0.164	0.313	0.486	0.317	0.422	0.207	0.378	0.343	0.367	-	0.016	0.016	0.010	0.008	0.011	0.016	0.015	0.013	0.026	0.036
HJBP	0.090	0.092	0.293	0.042	0.152	0.090	0.146	0.105	0.306	0.225	-	0.000	0.013	0.008	0.008	0.000	0.011	0.013	0.020	0.030
HRPR	0.317	0.348	0.549	0.208	0.354	0.083	0.148	0.159	0.353	0.317	-0.131	-	0.013	0.008	0.008	0.000	0.011	0.013	0.020	0.030
HNGP	0.270	0.381	0.582	0.262	0.484	0.059	0.111	0.083	0.278	0.172	0.183	0.205	-	0.005	0.008	0.013	0.011	0.010	0.020	0.030
HAKL	0.396	0.476	0.647	0.359	0.500	0.111	0.167	0.111	0.444	0.310	0.225	0.242	-0.091	-	0.003	0.008	0.006	0.008	0.018	0.028
HPNE	0.444	0.484	0.456	0.381	0.578	0.075	0.267	0.190	0.364	0.392	0.228	0.246	0.095	0.133	-	0.008	0.006	0.005	0.018	0.028
HHYD	0.498	0.495	0.643	0.417	0.548	0.381	0.606	0.556	0.620	0.532	-0.076	-0.037	0.588	0.567	0.567	-	0.011	0.013	0.020	0.030
HRCR	0.200	0.352	0.590	0.212	0.472	0.075	0.083	0.128	0.364	0.173	0.162	0.212	-0.056	0.133	0.222	0.419	-	0.008	0.021	0.031
HBGK	0.402	0.409	0.551	0.441	0.644	0.304	0.619	0.542	0.505	0.400	0.333	0.508	0.448	0.571	0.472	0.320	0.472	-	0.023	0.033
HGBL	0.264	0.338	0.426	0.290	0.356	0.178	0.306	0.275	0.322	0.282	0.215	0.278	0.194	0.262	0.264	0.453	0.264	0.345	-	0.023
HCBT	0.467	0.483	0.501	0.505	0.511	0.649	0.557	0.519	0.550	0.470	0.422	0.497	0.507	0.542	0.556	0.559	0.536	0.561	0.298	-

F_{ST} values in bold are significant after sequential Bonferroni correction ($P < 0.05$).

clade, and thus seem to have an ancestral difference from the populations considered in this study. The phylogeny also revealed that *Heliothis peltigera* is basal to *Helicoverpa assulta*, which in turn basal to *H. armigera*. All the populations also displayed a large number of mitochondrial haplotypes for the COI gene, of which 13 were shared and 26 unique (Fig. 3). Of the total shared haplotypes, haplotype 14, 18, 19 and 35 were highly common and shared by many locations or countries, while remaining were at least shared by two populations. However, the COI haplotype network was complex and there was no obvious pattern regarding the distribution of observed haplotypes in terms of geographical locations of the studied discrete populations.

Thus, characterization of different geographic populations of *H. armigera* from India based on COI gene indicated that these populations were not homogenous and maximum sequence divergence was up to four percent. However, Hebert *et al.* (2010) suggested that divergence of more than five percent in the COI gene amplicon depicts the likely occurrence of a new species in Lepidoptera. So the genetic divergence existing among the Indian populations of *H. armigera* is not high enough to delineate them as cryptic species, but the possibility of the existence of subspecies cannot be ruled out, as the intraspecific divergence is substantially higher than the postulated level of <3 percent as per Hebert *et al.* (2003). The phylogenetic tree also showed the presence of all the studied Indian populations into a common major clade, and this again strongly supported the single

species status of *H. armigera* in the country. However, clustering of populations into separate sub clades indicated about the existence of strong variability among them. All these sub clades were complex, as they included populations from geographically distant locations, thereby suggesting high gene flow among the populations in the region. The high migratory potential of the adult moths of this insect (Nibouche *et al.*, 1998) might have resulted in an intermingling of populations from different locations, as also observed in the complex haplotype network.

The presence of cryptic species of this insect in India was also not supported by Behere *et al.* (2007) based on the analyses of partial COI gene from 90 *H. armigera* specimens collected from northern, central and southern parts of the country. However, Asokan *et al.* (2012) indicated the possibility of emerging host-associated genetic differences in Indian populations of *H. armigera*. In this study also, it was apparent that there were a small number of populations particularly from the South Zone of the country that was significantly structured from all other sampled populations. High genetic differentiation of such populations may be the result of natural barriers that limited gene flow by blocking the spread of this mobile insect northward towards other zones. Indian *H. armigera* also showed high intra-population haplotype diversity. This indicates that it has a strong adaptability to varied environments. The occurrence of several unique haplotypes can be attributed to different eco-environmental conditions and vegetation patterns in India, along with wide distribution range of

The molecular evidences presented in this study thus seem to confirm substantial subspecific level variations among different geographic populations of *H. armigera* in India. Existence of such strong genetic variability within Indian *H. armigera* is the actual reason because of which it is exhibiting variability in its physiological characters and thereby differential

Fig. 3 : Median-joining haplotype network of *H. armigera* based on mitochondrial COI gene. Here, each circle (node) represents a haplotype and its area is proportional to haplotype frequency. Colours within the nodes refer to *H. armigera* sampling regions. Small red dots represent median vectors (hypothetical extinct or unsampled haplotypes).

response to various selection pressures. However, there is no existence of any unidentified cryptic species of *H. armigera* in the country. The findings of the present study strengthen information concerning the population structure of *H. armigera* in India and will also be helpful in developing appropriate integrated management strategies for this pest in the country.

Add-on Information

Author's contribution : **S. Chakravarty:** Contribution: Collected the insect samples, performed the experiments, data analysis, and prepared the first draft of the manuscript; **K. G. Padwal:** Helped in data analysis and interpretation, and edited the manuscript draft; **C.P. Srivastava:** Designed the study, supervised the experiments, and evaluated and finalized the manuscript draft.

Research content : The research content of manuscript is

original and has not been published elsewhere.

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