https://doi.org/10.33307/entomon.v48i1.839
ENTOMON 48(1): 11-18 (2023)
Article No. ent. 48102

Genetic diversity of cotton *Pectinophora gossypiella* (Saunders) (Lepidoptera, Gelechiidae) population inferred from mitochondrial *COI* sequences

I. Padma-Shree*, M. Muthuswami*[1], N. Manikanda Boopathi[2], K. Senguttuvan and S. Rajeswari[3]

Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India.
[1]Registrar office, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India.
[2]Department of Plant Biotechnology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India.
[3]Department of Cotton, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India.
Email: padhushree1996@gmail.com; mmsagricbe@gmail.com; nmboopathi@tnau.ac.in; senguttuvanphd@gmail.com; rajisundar93@gmail.com

ABSTRACT: The objective was to identify different haplotypes network of pink bollworm and their distribution in south India using the partial mitochondrial *COI* sequence of pink bollworm populations. Genetic diversity analysis exhibited the presence of 5 haplotypes in *COI gene* sequences, and among them, Hap_1 was the most common and present in 17 populations. Distributions of pairwise differences obtained with partial *COI* data from the overall populations were unimodal, suggesting population expansion. A significant neutrality test based on Tajima's D, Fu and Li's D and F test presented a haplotype network with multiple haplotypes. The acceptance of the neutrality test with significant positive values validated the theory of demographic expansion in cotton pink bollworm populations with respect to *COI*. The pink bollworm has a population genetic diversity among the investigated populations collected from south India. © 2023 Association for Advancement of Entomology

KEYWORDS: Haplotype, unimodal, neutrality test, demographic expansion

INTRODUCTION

Cotton is the most important cash crop in India and is used for industrial purposes all over the world (Keerthivarman *et al.,* 2022). The cotton crop is attacked by many species of insects at different stages of crop growth. The pink bollworm, *Pectinophora gossypiella* (Saunders) has become a significant production constraint on cotton production in India. The pest has developed multi-fold resistance to *Cry* toxins in many Indian populations and most of them have developed multifold resistance to *Cry 1 Ac* and *Cry 1Ac* + *Cry 2 Ab* toxins. Year-round cultivation of long-duration cotton hybrids on a large scale has a pronounced impact on the pink bollworm incidence (Prasada Rao, 2022). Mitochondrial DNA is widely used in taxonomy and systematics to explore the phylogenetic relationships of insects (Simon *et al.,* 1994; Boykin *et al.,* 2006) owing to their maternally inherited and well conserved genomic segments.

* Author for correspondence

© 2023 Association for Advancement of Entomology

As it evolves in a nearly neutral fashion, it reflects the divergence times, which can be used as a robust marker for determining genetic relationships and geographical studies (Bermingham and Lessios, 1993; Armstrong and Ball, 2005; Galtier *et al.*, 2009; Prabhakar *et al.*, 2013). Further, the genetic constitution of a pest is vital in determining the capacity to tolerate adverse climatic conditions and adoption to new conditions (Hayden *et al.*, 2011).

Population genetic structure and genetic diversity define the level of adaptation of a population to environmental change and susceptibility to selection pressure (Mopper, 1996; Pauls *et al.*, 2013). Gene flow through dispersion and migration, which is responsible for determining genetic variation leads to the evolution of local populations (Kremer *et al.*, 2012). In some lepidopteran species, the genetic diversity and structure were reported to be related to the migration capacity (Chen and Dorn, 2010; Men *et al.*, 2013). Studies on population structure and genetic diversity of pink bollworm (PBW) have been explored more in Asiatic countries such as India, Pakistan and China owing to the development of resistance to Bt cotton by PBW populations (Liu *et al.*, 2010; Sridhar *et al.*, 2017). Liu *et al.* (2009) studied the population genetic structure of Chinese PBW using mitochondrial *COI* and *Nad4* and found extremely low genetic variability among all populations examined. However, sequence variation in the *Nad4* region differentiated the Chinese populations from the Pakistani and American populations. Haplotypes and differentiation in PBW populations of China were identified using piggy Bac-like elements (Wang *et al.*, 2010). Sridhar *et al.* (2017), based on the analysis of the pink bollworm population from 19 districts, found that the pink bollworm population in India exhibited a low level of genetic diversity, and based on haplotype diversity results, they also opined that the populations might be experiencing population expansion; However, they could not provide the evidence through neutrality tests owing to the small population size. Thus the present investigation was designed to analyze the genetic diversity and distribution of *P. gossypiella* from selected populations using the *mitochondrial cytochrome oxidase I (mt COI)* gene.

MATERIALS AND METHODS

Pink bollworm samples were collected from the major cotton-growing tracts of Tamil Nadu *viz.* Coimbatore, Srivilliputhur, Veppathanttai and Salem were used. The collected larvae were stored in Eppendorf tubes with 70 per cent ethanol @ -20°C before DNA extraction. The genomic DNA was extracted from pink bollworm larvae using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle, 1991) with necessary modifications. Homogenization of larvae with 600 ìl of CTAB buffer (CTAB buffer: 1 mM Tris HCl; 0.5 mM EDTA; 5 mM NaCl; 2 % beta-mercaptoethanol, 2 % CTAB and pH 8.0) and made up to 1 ml with CTAB buffer. The mixture was incubated for 30 - 60 minutes at 65°C. Then treated with 500 ìl phenol-chloroform-isoamyl alcohol (25:24:1) and centrifuged at 12000 rpm for 10 minutes. The supernatant was transferred to a fresh Eppendorf tube containing 300 ìl chloroform-isoamyl alcohols (24:1) and the mixture was centrifuged at 12000 rpm for 10 min. Again, the supernatant was transferred to the fresh Eppendorf tube containing 250 to 500 ìl of isopropanol, for precipitation. The pellets were recovered by centrifugation @ 12000 rpm for 10 minutes and washed with 70 per cent ethanol and re-suspended in 30 - 50 ìl DEPC treated water. The quality and quantity of DNA were assessed using a NanoDrop spectrophotometer (Thermos Scientific, USA) and 0.8 per cent agarose gel.

PCR amplification and sequencing: The mitochondrial genes are inherited maternally and show sufficient diversity among the insect species. Hence, the primers (Table 1) which amplify the mitochondrial genes *viz*., *cytochrome c oxidase subunit I* was employed to generate PCR products. PCR analyses were performed in 25 ìl total reaction volume using 1 ìl of each forward (LCO1490) and reverse (HCO2198) primer. Polymerase Chain Reaction (PCR) was carried out in a thermal cycler with the following cycles: 94°C for 4 minutes as initial denaturation followed by 35 cycles of 94°C for 30 seconds, 48°C for 45 seconds, 72°C for 45 seconds and 72°C for 20 minutes as a final extension.

Table 1. Details of the COI primers used

No	Primer	Sequence (5'-3')	Tm*	Reference
		***COI* Primers**		
1	LCO1490	GCTCAACAAATCATAAGATATTGG	61.2°C	Folmer *et al.,* 1994
2	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	66.9°C	Folmer *et al.*, 1994

The amplified products were resolved in 1.5 per cent agarose gel, stained with ethidium bromide (10 ìg ml^{-1}) and visualized in a gel documentation system (Asokan *et al.,* 2011). The amplicons were commercially sequenced bidirectionally using the SANGER sequencing method (@ Biokart India Pvt. Ltd., Bangalore). The sequences were characterized using bioinformatics tools such as BLAST to check their homology. The sequences including all of the geographic populations were submitted to GenBank repositories and registered with accession numbers (Jiang *et al.,* 2014).

Phylogenetic analysis: The phylogenetic analysis based on the maximum likelihood (ML) method was performed using MEGA X version 7.0.9 (Kumar *et al.*, 2018) for investigating the degree of consistency of mutation patterns in different regions of India.

Data analysis for genetic divergence and haplotype distribution: Mitochondrial *COI gene* sequences were edited using Bio edit (Hall, 1999) and aligned using the ClustalW program (Thompson *et al.*, 1994) using MEGA X (Kumar *et al.*, 2018). Descriptive statistics on the number of haplotypes (H), haplotype diversity (Hd), variance and standard deviation of haplotype diversity were calculated using DnaSP version 5.10.01 (Librado and Rozas, 2009). Population history was inferred based on values of haplotype diversity (Hd) and nucleotide diversity (Pi). Small values of Hd and Pi (Hd < 0.5 and Pi < 0.005) represent population bottelneck or founder event by single or a few mtDNA lineages; high Hd and low Pi (Hd > and Pi < 0.005) represent population bottleneck folllowed by rapid population growth and accumualtion of mutations; low Hd and high Pi (Hd < and Pi > 0.005) represent divergence between geographically subdivided populations; large values of Hd and Pi (Hd > 0.5 and Pi > 0.005) represent large stable population with long evolutionary history or secondary contact between differentiated lineages To depict the evolutionary and geographical relationships among haplotypes, a minimum spanning haplotype network was constructed with PopART version 1.7 (Bandelt *et al.,* 1999).

Neutrality test and genetic differentiation: Fu and Li's D test; Fu and Li's F test and Tajima's D tests of neutrality index and genetic diferentiation are useful for demographic history information, with demographic expansion related to negative values and subdivided populations at equilibrium leading to positive values and hence they were also investigated using DnaSP version 5.10.01 for detecting the range of population expansions (Librado and Rozas, 2009).

RESULTS AND DISCUSSION

Phylogenetic analysis: The homology search of the sequences using NCBI BLAST matched with the *mt COI* sequences of pink bollworm in NCBI database, and the sequence similarity varied from 98 - 100 per cent. The nucleotide sequences were further aligned using MEGA version 7.0.9 and used for further analysis. The trimmed sequences were deposited in NCBI Gene Bank and accession numbers were obtained (Table 2). In these types of analyses, the nucleotide mismatch for each region was selected using the Tamura-Nei model. The evolutionary history was inferred using the Neighbour-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches. The evolutionary distances were computed using the Tamura 3 parameter method (Tamura, 1992) and were in the units of the number of base substitutions per site. This analysis involved 28 nucleotide sequences (Fig. 1).

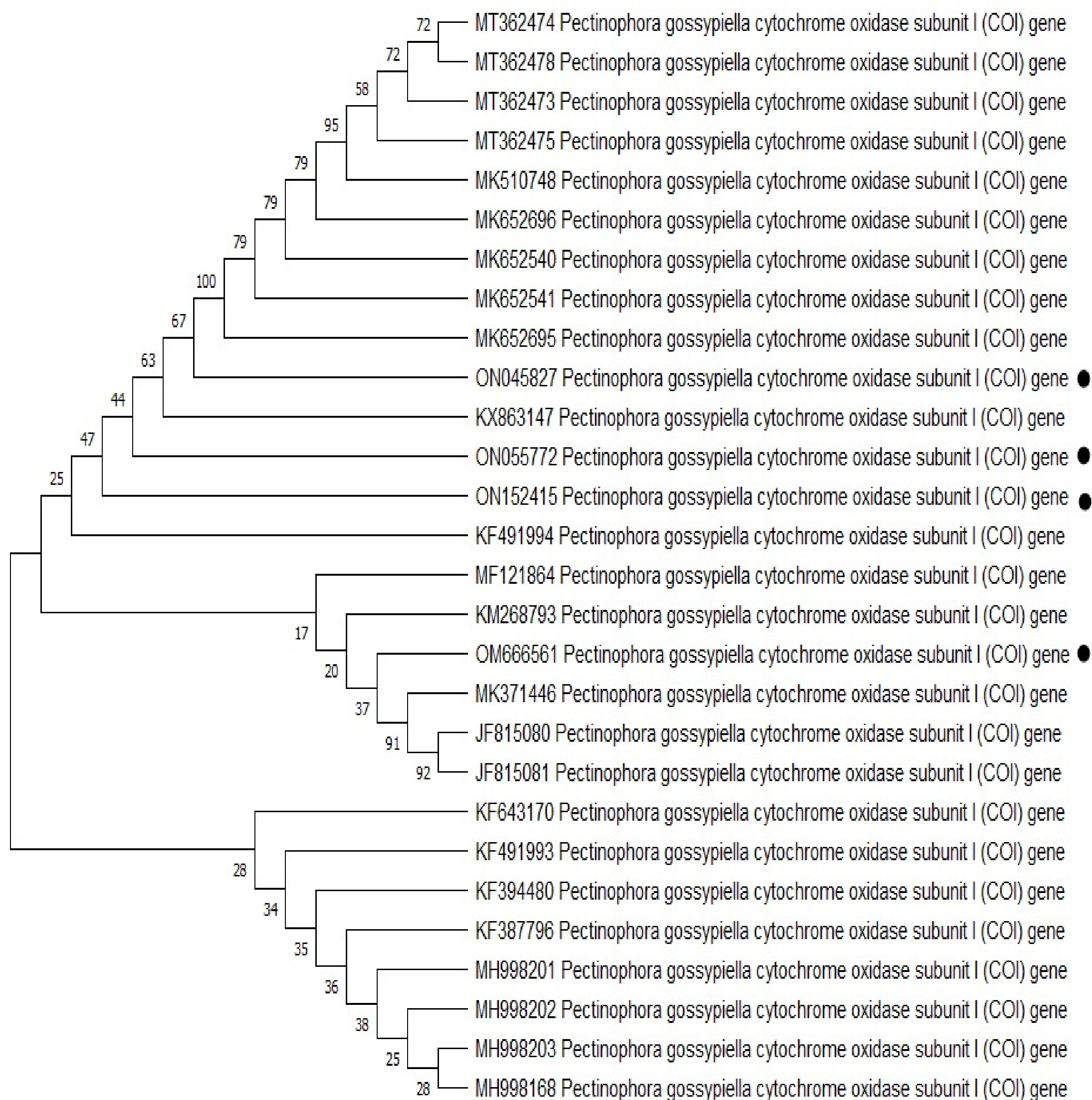

Fig. 1 Phylogenetic tree of the given *mt COI* gene sequences of *Pectinophora gossypiella*

The GenBank accession number with scientific names was included. Phylogenetic tree for the isolates of pink bollworm were constructed using the sequences of *COI* gene. The isolated *COI* genes were labelled as black spot (Table 2). Other sequences for the *COI* gene phylogenetic analysis were also obtained from the GenBank database (Table 3).

Table 2 *Pectinophora gossypiella mt COI* sequences developed and used in the current study

Collection	Location	Accession	*mt COI*
Coimbatore	11.0167N, 76.9350E	OM666561	638bp
Srivilliputhur	9.5045N, 77.6440E	ON055772	624bp
Veppanthattai	11.3516N, 78.8043E	ON045827	551bp
Salem	11.6560N, 78.4690E	ON152415	624bp

Table 3. *P. gossypiella mt COI* sequences from GenBank used in the current study

Location	Accession	*mt COI*
New Delhi, India	MH998201	651bp
New Delhi, India	MH998202	651bp
New Delhi, India	MH998203	651bp
Pakistan	KX863147	570pb
Pakistan	MK371446	687bp
USA	JF815080	688bp
USA	JF815081	688bp
Australia	KF387796	658bp
Australia	KF394480	658bp
USA	KF491994	642bp
USA	KF491993	658bp
Kenya	KF643170	658bp
Kenya	MF121864	658bp
Junagarh, India	MH998168	651bp
Bangalore, India	KM268793	658bp
Parbhani, India	MK652696	686bp
Parbhani, India	MK652695	686bp
Bangalore, India	MT362473	753bp
Guntur, India	MT362474	753bp
Raichur, India	MT362475	753bp
Warangal, India	MT362478	753bp
Akola, India	MK510748	711bp
Akola, India	MK652541	686bp
Akola, India	MK652540	686bp

Minimum spanning network among haplotypes: A minimum spanning network of haplotypes (Bandelt *et al.,* 1999) was generated using PopART version 1.7 (Fig. 2). The results confirmed five haplotypes *viz.* Hap_1, Hap_2, Hap_3, Hap_4 and Hap_5 for the selected populations of the pink bollworm. The network suggests that the most common haplotype of *COI gene* is Hap_1 followed by Hap_5, which was linked by Hap_3. These could be proposed as ancestral/ original haplotypes. Haplotypes were generally low in frequency with few populations formed and connected to nearby major haplotypes through several steps. The phylogenetic relationship studies revealed that most haplotypes were closely related to each other with the *COI* of the pink bollworm populations. The populations used in the study which were collected from Tamil Nadu falls under Hap_1 and Hap_2, which confirmed its association between the populations (Table 4).

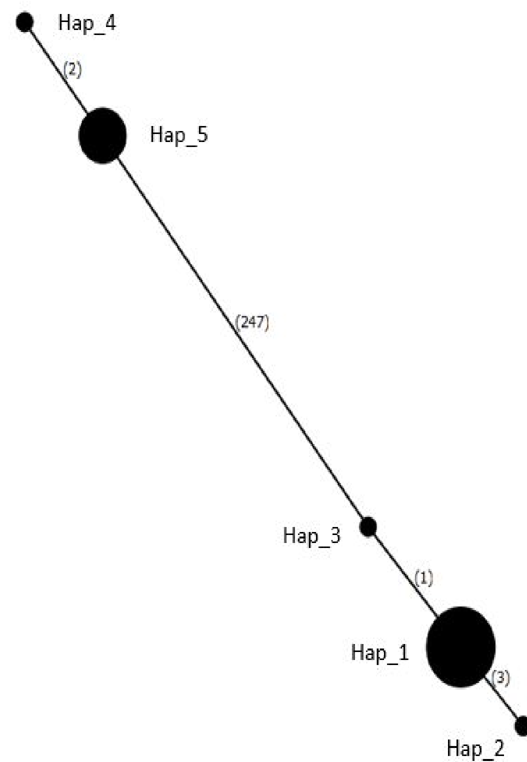

Fig. 2 *Haplotype network of Pectinophora gossypiella generated by DnaSP version 5.10.01 based on cytochrome c oxidase I sequences. A minimum spanning network was constructed using PopART version 1.7. Sequences with unverified species assignment and potential contaminants were not considered in the analyses. The circle size is proportional to the frequency of haplotypes. The numbers on the lines represent the number of nucleotide substitutions between linked haplotypes.*

Table 4. Comparison of the outputs of the presented identification tool and result of genetic analysis- Gene *COI*

Sequences	Haplotype - sequence analysis
Hap_1	OM666561_*Pectinophora gossypiella*
	ON152415_*Pectinophora gossypiella*
	ON055772_*Pectinophora gossypiella*
	MH998201_*Pectinophora gossypiella*
	MH998202_*Pectinophora gossypiella*
	KX863147_*Pectinophora gossypiella*
	MK371446_*Pectinophora gossypiella*
	JF815080_*Pectinophora gossypiella*
	JF815081_*Pectinophora gossypiella*
	KF387796_*Pectinophora gossypiella*
	KF394480_*Pectinophora gossypiella*
	KF491994_*Pectinophora gossypiella*
	KF491993_*Pectinophora gossypiella*
	KF643170_*Pectinophora gossypiella*
	MF121864_*Pectinophora gossypiella*
	MH998168_*Pectinophora gossypiella*
	KM268793_*Pectinophora gossypiella*
Hap_2	ON045827_*Pectinophora gossypiella*
Hap_3	MH998203_*Pectinophora gossypiella*
Hap_4	MK652696_*Pectinophora gossypiella*
Hap_5	MK652695_*Pectinophora gossypiella*
	MT362473_*Pectinophora gossypiella*
	MT362474_*Pectinophora gossypiella*
	MT362475_*Pectinophora gossypiella*
	MT362478_*Pectinophora gossypiella*
	MK510748_*Pectinophora gossypiella*
	MK652541_*Pectinophora gossypiella*
	MK652540_*Pectinophora gossypiella*

Population level diversity: Genetic diversity parameters including the number of haplotypes (5), haplotype diversity (0.566 Hd), nucleotide diversity (Pi 0.21625), variance of haplotype diversity (0.00631), and standard deviation of haplotype diversity (ì 0.079) that were determined with DnaSP version 5.10.01 suggested that they represented the large stable population with long evolutionary history or secondary contact between differentiated lineages.

Demographic history analysis: Tajima's D test (2.88869, $P < 0.01$**), Fu and Li's D test (1.82374, $P < 0.02$**), and Fu and Li's F test (2.57145, $P < 0.02$**), for *COI gene* in populations of cotton pink bollworm revealed high significance. Neutrality tests were accepted for all populations with significant positive values which confer to the hypothesis of past population expansion events. Three neutrality tests were performed and values were positive for populations of *COI,* indicating there was no excess of mutations which favours population expansion or growth. Thus, the results obtained in this study accept the hypothesis of neutral evolution for the investigated cotton PBW population and showed the demographic history among them.

A haplotype is a set of DNA variations (polymorphisms such as SNPs and InDels) adjacent to one another at the same locus that tend to be inherited together. This set of alleles is often referred to as linked polymorphisms (Durbin and Lathrop, 2010; Li *et al.,* 2010). The studies revealed that total of 5 unique haplotypes while 17 individuals at Hap_1 and 8 individuals at Hap_5 were inferred from mitochondrial *COI* sequences. It has been supported by Sridhar *et al.* (2017) who found that 12 (15.18 %) haplotypes in 79 individuals were distributed in 19 populations in Indian subcontinent. The first most predominant haplotype was found in 143 individuals and the second most predominant haplotype was shared with 32 individuals as confirmed with the haplotype network shared by populations across different zones.

This study, reported low mitochondrial DNA variations (0.566 Hd) in taxa that might have undergone severe bottlenecks or founder effects. These results have supported by Sridhar *et al.* (2017) who found 0.3028 Hd in overall populations of pink during 2011 - 2012 populations before they broke the resistance to both *Cry1Ac* and *Cry2Ab* in India. Similarly extremely low level of population genetic variation was also observed in the two mitochondrial regions (*COII and Nad4*) among the nine Chinese Pink Bollworm populations (Liu *et al.,* 2010) and such variations were attributed to invasion bottlenecks, which had subsequently strengthened by its non-migratory biology and the mosaic pattern of agricultural activities (Liu *et al.,* 2010). These

haplotype information can be categorized by its applications in the identification of pink bollworm as well as its adaptation to diverse ecological niches and mechanisms of insecticide resistance. Proper assimilation and understanding of the genetic diversity of an insect pest have been found essential to mitigate and improve its monitoring that further facilitating the implementation of need based management strategies (Naik *et al.,* 2020).

ACKNOWLEDGMENTS

The authors are thankful to the Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore and the Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore for the materials and support provided during the research work.

REFERENCES

Armstrong K.F. and Ball S.L. (2005) DNA barcodes for biosecurity: invasive species identification. Philosophical Transactions of the Royal Society B: Biological Sciences 360(1462): 1813–1823.

Asokan R., Rebijith K.B., Singh S.K., Sidhu A.S., Siddharthan S., Karanth P.K., Ellango R. and Ramamurthy V.V. (2011). Molecular identification and phylogeny of *Bactrocera* species (Diptera: Tephritidae). Florida Entomologist 94(4): 1026–1035.

Bandelt H., Forster P. and Rohl A. (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16(1): 37–48.

Bermingham E. and Lessios H.A. (1993) Rate variation of protein and mitochondrial DNA evolution as revealed by sea urchins separated by the Isthmus of Panama. Proceedings of the National Academy of Sciences 90(7): 2734–2738.

Boykin L.M., Shatters R.G., Hall D.G., Burns R.E. and Franqui R.A. (2006) Analysis of host preference and geographical distribution of *Anastrepha suspensa* (Diptera: Tephritidae) using phylogenetic analyses of mitochondrial cytochrome oxidase I DNA sequence data. Bulletin of entomological research 96(5): 457–469.

Chen M.H. and Dorn S. (2010) Microsatellites reveal genetic differentiation among populations in an insect species with high genetic variability in dispersal, the codling moth, *Cydia pomonella* (L.)(Lepidoptera: Tortricidae). Bulletin of Entomological Research 100(1): 75–85.

Doyle J. (1991) DNA Protocols for Plants. In: Molecular Techniques in Taxonomy: Vol. 57, Springer, Berlin, Heidelberg, pp 283–293. https://doi.org/10.1007/978-3-642-83962-7_18

Durbin R.M. and Lathrop M. (2010) A map of human genome variation from population-scale sequencing. Nature 467(7319): 1061–1073.

Folmer O., Black M., Hoeh W., Lutz R. and Vrijenhoek R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates, Molecular marine biology and biotechnology 3: 294–299.

Galtier N., Nabholz B., Glemin S. and Hurst G.D.D. (2009) Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Molecular ecology 18(22): 4541–4550.

Hall T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic acids symposium series Vol. 41, London, Information Retrieval Ltd. pp 1979–2000.

Hayden E.J., Ferrada E. and Wagner A. (2011) Cryptic genetic variation promotes rapid evolutionary adaptation in an RNA enzyme. Nature 474(7349): 92–95.

Jiang F., Li Z. H., Wu J.J., Wang F.X. and Xiong H.L. (2014) A rapid diagnostic tool for two species of *Tetradacus* (Diptera: Tephritidae: *Bactrocera*) based on species specific PCR. Journal of Applied Entomology 138(6): 418–422.

Keerthivarman K., Mahalingam L., Premalatha N., Boopathi N.M., Senguttuvan K. and Manivannan A. (2022) Combining ability and heterosis studies in cotton (*Gossypium hirsutum* L.) for yield and fibre quality traits. Electronic Journal of Plant Breeding 13(2): 697–704.

Kremer A., Ronce O., Robledo Arnuncio J.J., Guillaume F., Bohrer G., Nathan R., Bridle J.R., Gomulkiewicz R., Klein E.K., Ritland K. and Schueler S. (2012) Long distance gene flow and adaptation of forest trees to rapid climate change. Ecology letters 15(4): 378–392.

Kumar S., Stecher G., Li M., Knyaz C. and Tamura K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution 35(6): 1547.

Li Y., Willer C.J., Ding J., Scheet P. and Abecasis G.R. (2010) MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genetic epidemiology, 34(8): 816–834.

Librado P. and Rozas J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25(11): 1451–1452.

Liu Y. D., Wu K.M. and Guo Y.Y. (2009) Population structure and introduction history of the pink bollworm, *Pectinophora gossypiella*, in China. Entomologia experimentalis et applicata 130(2): 160–172.

Liu Y., Hou M. and Wu K. (2010). Genetic variation of mitochondrial DNA in Chinese populations of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). Environmental entomology 39(4): 1344–1351.

Men Q.L., Chen M.H., Zhang Y.L. and Feng J.N. (2013) Genetic structure and diversity of a newly invasive species, the codling moth, *Cydia pomonella* (L.)(Lepidoptera: Tortricidae) in China. Biological invasions 15(2): 447–458.

Mopper S. (1996). Adaptive genetic structure in phytophagous insect populations. Trends in Ecology and Evolution 11(6): 235–238.

Naik V., Pusadkar P.P., Waghmare S.T., Kranthi S., Kumbhare S., Nagrare V.S., Kumar R., Prabhulinga T., Gokte-Narkhedkar N. and Waghmare V.N. (2020) Evidence for population expansion of cotton pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) in India. Scientific reports 10(1): 1–11.

Pauls S.U., Nowak C., Balint M. and Pfenninger M. (2013) The impact of global climate change on genetic diversity within populations and species. Molecular ecology 22(4): 925–946.

Prabhakar C.S., Sood P., Mehta P.K. and Sharma P.N. (2013) Population genetic structure of the pumpkin fruit fly, *Bactrocera tau* (Walker) (Diptera: Tephritidae) in Himachal Pradesh, India. Biochemical systematics and ecology 51: 291–296.

Prasada Rao G.M.V. (2022) Indian scenario on the occurrence of a dreaded insect pest Pink bollworm, *Pectinophora gossypiella* on Bt cotton-A review. Journal of Environmental Biology 43(1): 11–19.

Saitou N. and Nei M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4(4): 406–425.

Simon C., Frati F., Beckenbach A., Crespi B., Liu H. and Flook P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the entomological Society of America 87(6): 651–701.

Sridhar J., Naik V.C.B., Ghodke A., Kranthi S., Kranthi K.R., Singh B.P., Choudhary J.S. and Krishna M.S.R. (2017) Population genetic structure of cotton pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) using mitochondrial *cytochrome oxidase I (COI)* gene sequences from India. Mitochondrial DNA Part A 28(6): 941–948.

Tamura K. (1992) Estimation of the number of nucleotide substitutions when there are strong transition - transversion and G+ C-content biases. Molecular Biology and Evolution 9(4): 678–687.

Thompson J.D., Higgins D.G. and Gibson T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22(22): 4673–4680.

Wang J., Miller E.D., Simmons G.S., Miller T.A., Tabashnik B.E. and Park Y. (2010) Piggy Bac like elements in the pink bollworm, *Pectinophora gossypiella*. Insect Molecular Biology 19(2): 177–184.

(Received September 22, 2022; revised ms accepted January 21, 2023; published March 31, 2023)