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Genetic Diversity of Army worm, *Spodoptera mauritia* Isolated from Kerala, India

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The army worm *Spodoptera mauritia* is one of the major pests of paddy which is widely distributed in the Indian subcontinent, East and southern Asia and in the Australian region. Generally the army worms infest paddy crops of less than 20-25 days old. They are gregarious, defoliating the paddy and move from one field to other in large number like an army. The classification of *Spodoptera* species is mainly based on the structure of the male genitalia, antenna and the colour pattern of the wing. Here we report the partial coding sequence of cytochrome oxidase sub-unit I (COI) sequence of army worm isolated from Kerala, India which is identical to that isolated from Japan. This study highlights the geographical distribution and genetic diversity of army worm in paddy cultivating countries.

Key words: Cytochrome oxidase subunit I, geographical distribution, *Spodoptera mauritia*, Divergence.

The Army worm, *Spodoptera mauritia* is a gregarious pest which causes severe damage to rice. The larvae of army worm defoliate the rice crop and move from one field to another field in large number like an army devastating the entire crop (Krishnaiah *et al*., 2008). The pest is widely distributed in Asia and Australia. In India army worm infestation were reported from Kerala, Tamilnadu, Orissa, Bihar, Jharkhand and Chhatisgarh (David and Ananthakrishanan, 2004, Tanwar *et al*., 2010)

The classification of *Spodoptera* species is mainly based on the structure of the male antenna and the colour pattern of the wing (Hampson, 1920). The colour pattern of the *Spodoptera* species shows variations that may leads to the wrong identification. The male genitalia give the reliable taxonomic character to identify this species (Chatterjee, 1967). The genetic structure of an organism can provide wealth information to for identification and to study variations within the species and between the species. The genetic structure of several *Spodoptera*n species have been reported in many studies (Machado *et al*., 2008; Wan *et al*., 2011; Nagoshi *et al*., 2011). The partial coding

sequence of Cytochrome oxidase sub unit I of *S. mauritia* isolated from Japan and Reunion Islands were reported in the NCBI (GenBank Accession No. AB733407, AB733409, AB733408, HQ177382, HQ177383 and HQ177384). The COI sequence of *S. mauritia* isolated from Japan showed 3.1% divergence for that isolated from Reunion. The studies on genetic structure of *Spodoptera* species from India are limited. But there is no report on the genetic structure analysis of *S. mauritia* from India, even though its cause's major economic loss. Hence In this study we have described the genetic structure of *S. mauritia* isolated from Kerala, India and also made a comparative genetic structure analysis of *S. mauritia* isolated from Kerala, India with geographically isolated populations.

Materials and Methods

Different life stages of *S. mauritia* were collected from the rice field of Kerala, India and sample were stored at -20^{0}C until the DNA was extracted. The genomic DNA was isolated using GeNei Ultrapure Mammalian Genomic DNA Prep Kit (Bangalore GeNei, Bangalore) as per the Manufacturer's instruction. The 5' end of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the forward primer with DNA sequence 5' CAT TGG AGA TGA CCA AAT TTA TAA TG -3' and reverse primer with DNA sequence 5'- TGA AAT TAA TCC AAA TCC AGG TAA A-3'. The 25 µl PCR reaction mixture consisted of 2 nanogram of genomic DNA (1 µl), 1 µl each forward and reverse primers at a concentration of 10 µM, 2.5 µl of dNTPs (2 mM), 2.5 µl 10X reaction buffer, 0.20 µl Taq polymerase (5 U/µl) and 16.8 µl H_2O. The PCR profile consisted of an initial denaturation step of 3 min at 95^{0}C, followed by 30 cycles of 10 sec at 95^{0}C, 45 sec at 45^{0}C and 45 sec at 72^{0}C and ending with a final phase of 72^{0}C for 3 min. The PCR product was column purified using Mo Bio UltraClean PCR Clean-up Kit (Mo Bio Laboratories, Inc. California) as per the manufacturer's instructions.

The purified PCR product was sequenced from both ends using the forward and reverse primers using the Sanger's sequencing method at SciGenom Laboratories Ltd., Cochin. The forward and reverse sequences were assembled by using ClustalW after removing the forward and reverse primers and the consensus was taken for the analysis. The phylogenetic analysis was done using MEGA5 software.

Results and Discussion

The Rice army worm *Spodoptera mauritia* under the family Noctuidae is one of the most serious paddy pests of Kerala. In this study, we have collected various life stages of *S. mauritia* from the paddy fields of Kerala. The dark brown adult was 1.7mm in length. The eggs were spherical and creamy in colour and they were covered with gray hairs. The freshly hatched larvae were 2mm in length with light green with yellowish white lateral and dorsal stripes. The pupa was dark brown in colour and measures 15 mm in length, having two slender apical spines. The mature larva was 4 cm in length, light green in colour and two rows of C-shaped black spots were observed along the backs.

The partial coding sequence of COI gene of *S. mauritia* was PCR amplified using the forward primer with DNA sequence of 5' CATTGGAGATGACCAAATTTA TAATG 3' and the reverse primer with DNA sequence of 5'TAA ACT TCA GGG TGA CCA AAA AAT CA 3'. The PCR amplification of partial mitochondrial COI gene of *S. mauritia* yielded a single product with about 600 bp in size. The sequence obtained after removing the primers used for PCR amplification was submitted to GenBank (GenBank Accession No. KC601856).

The nucleotide composition analysis showed a similarity in the concentration of

each nucleotide in the COI sequence of *S. mauritia* isolated from India and other geographically isolated population. Usage of nucleotides in each position of codon of *S. mauritia* isolated from India is similar to *S. mauritia* (GenBank Accession Nos. AB733407, AB733409, AB733408) isolated from Japan but it is different from *S. mauritia* from Reunion islands (GenBank Accession Nos. HQ177382, HQ177383, HQ177384, HQ177386). The *S. mauritia* of Reunion islands showed 0.7-1% variation in the usage of nucleotides in each position of codon of *S. mauritia* isolated from India, except in the third position of codon.

The sequence divergence analysis clearly depicts the evolutionary divergence between the *S. mauritia* isolated from India and other geographically isolated populations. The COI sequence of *S. mauritia* isolated from India is identical with that isolated from Japan (GenBank Accession No. AB733407, AB733409, AB733408). The *S. mauritia* isolated from India has 0.52% divergence with *S. mauritia acronyctoides* (GenBank Accession Nos. HQ177386) isolated from Reunion Island and it showed more than 3% divergence with that of isolated from Reunion Islands (GenBank Accession Nos. HQ177382, HQ177383, HQ177384). Among other *Spodoptera* sp. used in this study *S. cilium, S. albula, S. cosmiodes* and *S. tritura* showed less divergence with *S. mauritia* isolated from India and *S. exigua* showed highest divergence (Table 1). The phylogeny analysis using Neighbor Joining method revealed that the *S. mauritia* evolved two clad. The *S. mauritia* isolated from India and Japan are aligned in a clad and *S. mauritia acronyctoides* (GenBank Accession No HQ177386) isolated from Reunion formed a sister clad of it. Other *S. mauritia* isolated from Reunion are arranged in the second clad. The *S. mauritia* isolated from India and Japan shared a common origin. Among the other *Spodoptera* sp. used in this study *S. triturata* is the nearest relative of *S. mauritia* isolated from India and *S. exigua* is the distant relative (Fig.1).

Table 1. Evolutionary divergence between COI sequences of the species *S. mauritia* isolated from Kerala, India with the different Sopodoptera species indentified from different geographical locations. The GenBank accession numbers are given in parenthesis.

S. No.	Name of Species	% of divergence
1	*S. mauritia* (AB733409) Japan	0.00%
2	*S. mauritia* (AB733408) Japan	0.00%
3	*S. mauritia* (AB733407) Japan	0.00%
4	*S. mauritia* acronyctoides (HQ177386) Reunion	0.52%
5	*S. mauritia* (HQ177384) Reunion	3.15%
6	*S. mauritia* (HQ177383) Reunion	3.15%
7	*S. mauritia* (HQ177382) Reunion	3.70%
8	*S. cilium* (JN988597)	7.40%
9	*S. cilium* (JN988598)	7.40%
10	*S. albula* (JQ567811)	7.68%
11	*S. cosmioides* (JF854738)	7.72%
12	*S. triturata* (HM892940)	7.81%
13	*S.* triturata (HM892616)	7.83%
14	*S. exempta* (DQ092375)	7.97%
15	*S. frugiperda* (HM136592)	8.01%
16	*S. frugiperda* (HM136591)	8.01%
17	*S. frugiperda* (HM136590)	8.01%
18	*S. cosmioides* (HQ571029)	8.01%
19	*S. albula* (JQ535527)	8.27%
20	*S. littoralis* (HM756074)	8.29%
21	*S. ornithogalli* (EU768964)	8.32%
22	*S. litura* (JQ064571)	8.55%
23	*S.* l*atifascia* (JQ577384)	8.84%
24	*S. pulchella* (HM756076)	9.08%
25	*S. pulchella* (HM756075)	9.08%
26	*S. androgea* (GU159412)	9.15%
27	*S.dolichos* (HM756088)	9.44%
28	*S. exempta* (DQ092376)	9.49%
29	*S. dolichos* (HM756086)	9.73%
30	*S.* e*ridania* (HM756083)	10.05%
31	*S. eridania* (JQ546665)	10.05%
32	*S. exigua* (GU707393)	10.68%
33	*S. exigua* (JF415658)	10.68%
34	*S. exigua* (HM914242)	10.68%

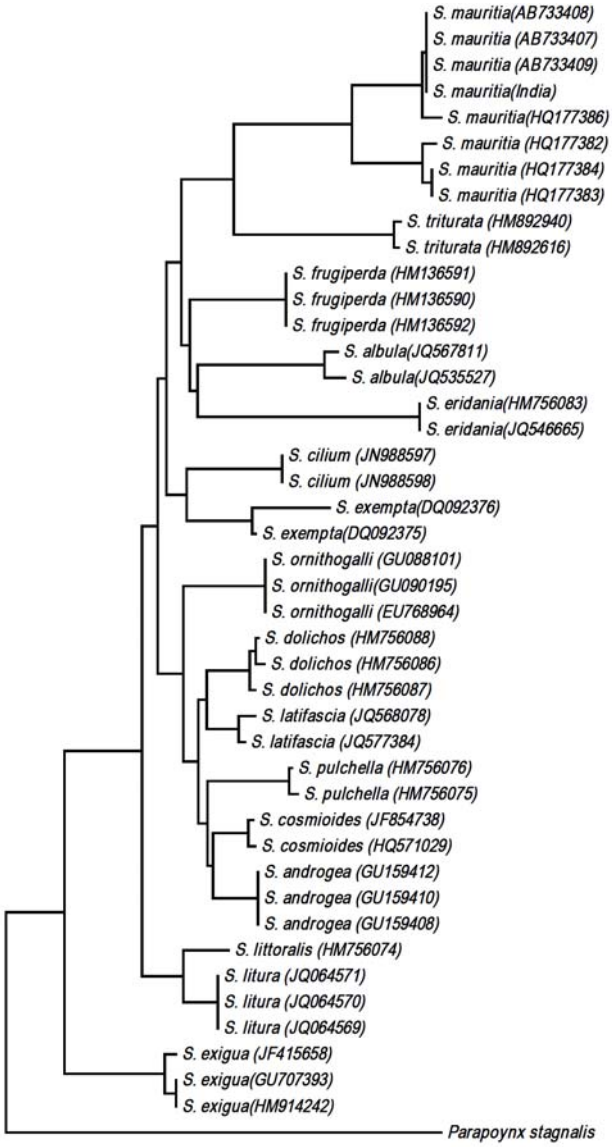

Fig. 2. Phylogenic relationship of *S. mauritia* isolated from Kerala, inferred by NJ tree method of MEGA5 software. The GenBank accession numbers are given in parenthesis.

DNA sequence based identification technique has revealed the morphological and ecological traits of many species during larval stages (Foltan 2005; Smith 2006; Hayashi and Sota 2010). According to Gurney *et al.* (2000) closely related species have 90% similarity in the standardized DNA sequence but distantly related species have less than 90% similarity in the same genes sequence. The COI sequence of *S. mauritia* showed close similarity within the species and considerable variation between the species.

The genetic structure analysis of COI sequence of *S. mauritia* indicates the similarity in the nucleotide composition and variation in the nucleotide usage in the each position of codon of COI gene of the different *S. mauritia* populations. Variation in

nucleotides in the codon of COI sequence revealed that the third position in the codon of *S. mauritia* is conserved compared to first and second position. In the first and second position the nucleotide **'T'** composition has high variation in the COI sequence of *S. mauritia* isolated from India and Reunion compared to other nucleotides and its COI sequence is highly biased to **AT**. The shift to **AT** compositional bias with low '**G**' in the sense and '**C**' in the template strand may have arisen through directed mutational pressure (Jermiin *et al.,* 1994).

The nucleotide divergence study and the phylogeny analysis also revealed the origin of *S. mauritia* isolated from India. Generally the genetic variation in the COI sequence of the same species will be very low averaging 0.43%, while in the different species of the same genus showed 18 fold higher sequence divergence averaging 7.7% (Hebert *et al.,* 2010). *S. mauritia* isolated from India showed 0-3.7% and 2% average intraspecific divergence with that of geographically isolated population. The geographical isolation may be the reason for this high divergence of the *S. mauritia* between the populations. The average interspecific divergence between the members of the *Spodoptera* genus was also found high (7%). Most of the members of genus *Spodoptera* are polyphagous and they can feed several plant families (Brown and Dewhurst, 1975; Pogue, 2002). The high interspecific divergence observed among the species of genus *Spodoptera* may be due to the polyphagy. Even though Japan is an island which is separated by over 5000KM from India, the *S. mauritia* isolated from India showed 100% similarity with that isolated from Japan. The similarity of *S. mauritia* of these geographically isolated populations (India and Japan) revealed the recent spreading of the *S. mauritia* to this area from a common origin and it also indicates the low mutation rate in the COI sequence of *S. mauritia* isolated from India and Japan.

The different population of *S. mauritia* has same concentration of each nucleotide in COI sequence and the COI sequence is highly biased towards the nucleotides **A** and **T**. But in the codon usage the concentration of each nucleotide in the each position of codon of may be varied depending upon the population. The nucleotide in the third position of the codon of the *S. mauritia* COI sequence has little chance for variation. The *S. mauritia* isolated from the Reunion has high divergence with Indian population and *S. exigua* is highly diverged from *S. mauritia*. Phylogeneticaly *S. triturata* is the nearest relative of *S. mauritia* among the *Spodoptera* species analysed.

References

Brown, E.S. and Dewhurst, C.F. 1975. The genus Spodoptera (Lepidoptera: Noctuidae) in Africa and the Near East. *Bull. Entomol. Res.,* 65: 221–262.

Chatterjee, S.N. 1967. The identity of *Spodoptera mauritia* Acronyctoides guenee, *Spodoptera pecten* Guenee and *Spodoptera abyssinia* Guenee (Lepidoptera: Noctuidae) based on a comparative study of the male and female genitalia, *Proc. Natl. Acad. Sci. India,* **35**: 45-62.

David, B.V. and Ananthakrishnan, T.N. 2004. The swarming caterpillar or armyworm, *Spodoptera mauritia*. In "*General and Applied Entomology" (Second Edition).* pp.1184. Tata McGraw-Hill Publishing Co. Ltd, New Delhi.

Foltan, P., Sheppard, S.K., Konvicka, M. and Symondson, W.O.C. 2005. The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators using PCR. *Mol. Ecol.,* **14**: 4147–4158.

Gurney, T. jr., Elbel, R., Ratnapradipa, D. and Brossard, R. 2000. "Introduction to the molecular phylogeny of insects. Tested

studies for laboratory teaching", (S. J. Karcher, Eds.), *Proceedings of the 21st Workshop/Conference of the Association for Biology Laboratory Education*, **21:** 63-79.

Hampson, G.F. 1920. Catalogue of the Lepidoptera Phalaena in the British Museum. 8.

Hayashi, M. and Sota, T. 2010. Identification of elmid larvae (Coleoptera: Elmidae) from Sanin District of Honshu, Japan, based on mitochondrial DNA sequences. *Entomol. Sci.*, **13**: 417–424.

Hebert, P.D.N., Deward, J.R. and Landry, J.F. 2010. DNA barcodes for 1/1000 of the animal kingdom. *Biol. Lett*., **6**: 359-362.

Jermiin, L.S., Graur, D., Lowe, R.M. and Crozier, R.H. 1994. Analysis of directional mutation pressure and nucleotide content in mitochondrial cytochrome b genes. *J. Mol. Evol*. **39**: 160-73.

Machado, V., Wunder, M., Baldissera, V.D., Oliveira, J.V., Fiúza, L.M. and Nagoshi, R.N. 2008. Molecular Characterization of Host Strains of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Southern Brazil. *Ann. Entomol. Soc. Am.*, **101**: 619-626.

Nagoshi, R.N., Brambila, J. and Meagher, R.L. 2011. Use of DNA barcodes to identify invasive armyworm, Spodoptera species in Florida. *J. Insect Sci*. **11**: 154

Pogue, M.G. 2002. A world revision of the genus Spodoptera Guenée (Lepidoptera: Noctuidae). *Mem. Am. Entomol. Soc*., **43**: 1-202.

Smith, M.A., Woodley, N.E., Janzen, D.H., Hallwachs, W. and Hebert, P.D.N. (2006). DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *PNAS,* **103**:3657–3662.

Tanwar, R.K., Anand Prakash, S.K., Panda, N.C., Swain, D.K., Garg, S.P., Singh, S., Kumar, S. and Bambawale, O.M. 2010. Rice swarming caterpillar (*Spodoptera mauritia*) and its management strategies, *Technical Bulletin 24*. National Centre for Integrated Pest Management, New Delhi.

Wan, X., Li, J., Kim, M.J., Park, H.C., Kim, S.S. and Kim, I. 2011. DNA Sequence Variation of the Tobacco Cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae), Determined by Mitochondrial A+T-rich Region and Nuclear ITS2 Sequences. *Biochem. Genet*., **49**: 760-787.