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Genetic variation of cotton bollworm, *Helicoverpa armigera* (Hübner) of South Indian cotton ecosystem using RAPD markers

Documenting the nature of genetic variation, magnitude and distribution is necessary for understanding the behaviour, response to selection pressure, structure and dynamics of different populations and management[1–5]. Availability of reliable polymorphic markers often limits the accurate estimation of genetic variation among individuals or different populations. Elucidation of genetic variation in geographical populations can be an important aspect to study the pest populations and their management[6]. Within an ecosystem, the extent of genetic variation between geographical populations depends on several factors, including gene flow between populations, host range and time since separation[7,8]. Genetic differences within and between geographic populations of an ecosystem are likely to be defined by the population fluxing patterns as influenced by various ecological factors in the immediate past and the historical pressures on the genome[9].

Usual DNA-based techniques such as Restriction Fragment Length Polymorphism (RFLP) through Southern hybridization and use of microsatellites are expensive; use of the latter is often hindered by lack of availability of DNA sequence information, though it has inherent advantage[10]. Polymerase Chain Reaction (PCR)-based Random Amplified Polymorphic DNA (RAPD) approach has been a handy and convenient alternative technique for investigations of genetic variation and genome mapping[11,12]. Because of the nature of primer sequences, RAPD analysis samples the genome more randomly than other methods and has been successfully employed in the construction of linkage maps[13–16]. Being simple and non-radioactive, the technique is quite sensitive and used to detect genetic variation in many organisms[17–21]. It has been extensively used for molecular fingerprinting[22–24], phylogenetic analyses[25,26], genetic mapping[27] and population diversity[18,28,29] analysis. The variation that can be accounted for, between and within populations through RAPD, appears to be unlimited. Yet, the dominance nature of these markers is a greater leveller and introduces subjectivity in understanding the structure of populations, where allelic frequencies of genes matter. Further, cyclic amplification of DNA being an extremely powerful technique, RAPD patterns are protocol-sensitive, which limits the cross comparison of information generated by this method with others.

Cotton bollworm, *Helicoverpa armigera* (Hübner) is a key pest of cotton and other crops in India and elsewhere, inflicting huge crop loss each year. Looking at its versatility in rapidly evolving resistance to almost all classes of insecticides and its ability to thrive on several hosts, there must be a strong genetic basis governing the behaviour of *H. armigera* in making it a serious pest on several crops. Thus, the understanding of genetic variation within and between geographical populations of *H. armigera* in the cotton ecosystem and genome-fluxing patterns, coupled with estimating resistance folds to each insecticide can expectedly help in pinning down the exact causes for such frequent outbreaks and versatility in evolving resistance to insecticides at a faster rate. Elucidation of gene statements responsible for insecticide resistance in *H. armigera* would bring more light in understanding the phenomenon and management of the problem. In the Indian context, a systematic and concerted effort to view the problem of insecticide resistance from this perspective is important.

We report in the present study, the genetic variability as revealed by RAPD in 12 geographical populations representing the entire South Indian cotton ecosystem. Cotton bollworms were collected during peak incidence from each of the 12 locations of the South Indian cotton ecosystem: Nanded, Nagpur and Parbhani (Maharashtra); Guntur, Madhira and Nalgonda (Andhra Pradesh); Raichur, Dharwad and Mysore (Karnataka); Coimbatore, Madurai and Kovilpatti (Tamil Nadu) (Figure 1). About 20 larvae for each location were randomly picked for isolation of genomic DNA separately. The larvae were desensitized using formalin swab, each larva was dissected and the gut contents were completely removed to avoid any contamination of plant DNA. Resulting skin and legs were used to prepare genomic DNA following modified CTAB method. DNA was further purified by phenol–chloroform treatment. In order to make a better representation of each location, equal amount of DNA from each of 20 larvae for each location was pooled and the resulting 12 bulked DNA samples were used for PCR–RAPD analysis. Bulked DNA was diluted to 20–40 ng/µl before actually being used

in PCR reactions. A set of 40 random decamer primers randomly selected from OPA, OPC, OPD and OPO kits obtained from Operon Technologies Inc., USA was tested across all the 12 DNA samples. PCR was carried out for each primer in 25 µl standard reaction mixture consisting of 40 ng of template DNA, 0.2 mM primer, 3.5 mM magnesium chloride, 1x Bangalore Genie buffer, 0.6 U *Taq* polymerase (Bangalore Genie Pvt Ltd) and 200 mM each dATP, dTTP, dGTP and dCTP (Bangalore Genie Pvt Ltd). PCR amplification was carried out on an Eppendorf Master gradient cycler (Eppendorf AG, Germany) in 0.5 ml micro-centrifuge tubes. Mineral oil was not added to the reaction mixture as the thermal cycler had hot-lid facility. PCR started with a 2-min initial denaturation at 94°C followed by 40 cycles of 1 min at 94°C for denaturation, 1 min at 36°C for annealing, 2 min at 72°C for extension and ended with a final 10 min extension at 72°C. These reaction products were kept overnight at 4°C or for several days at –70°C (if required) prior to electrophoresis on 1.3% agarose gels at 5 V/cm for 3 h, which resolved DNA fragments ranging from 100 bp to 3 Kb. All the 40 primers were tested at least twice for the reproducibility of banding pattern. A set of 25 primers, viz. OPA 01, OPA 02, OPA 03, OPA 04, OPA 05, OPA 16, OPA 17, OPA 18, OPD 01, OPD 02, OPD 03, OPD 07, OPD 08, OPD 11, OPD 12, OPD 13, OPO 03, OPO 06, OPO 07, OPO 11, OPO 12, OPO 15, OPO 16, OPO 18 and OPO 20 producing reproducible banding patterns were selected for the present study. All the bands, in the range of resolution were scored, except for very faint and ghost bands. The gel pictures acquired through a gel documentation system into a computer were processed and scored to get binary data.

The presence/absence data (1, 0) matrix was analysed using the standard procedure in NTSYS Pc2 package. Similarity matrix was computed for each individual population. Genetic distance or similarity was determined by Jacquard similarity[30,31]. The resultant similarity matrix was used to generate a tree by UPGMA (unweighted pair group method with arithmetic average) in NTSYS Pc2 software package.

All the 25 gels resulting from short-listed primers had maximum number of clear and scorable amplicons in each DNA sample with few ghost or minor bands, which were ignored. Sample gel resulting from OPA 16 random primer across

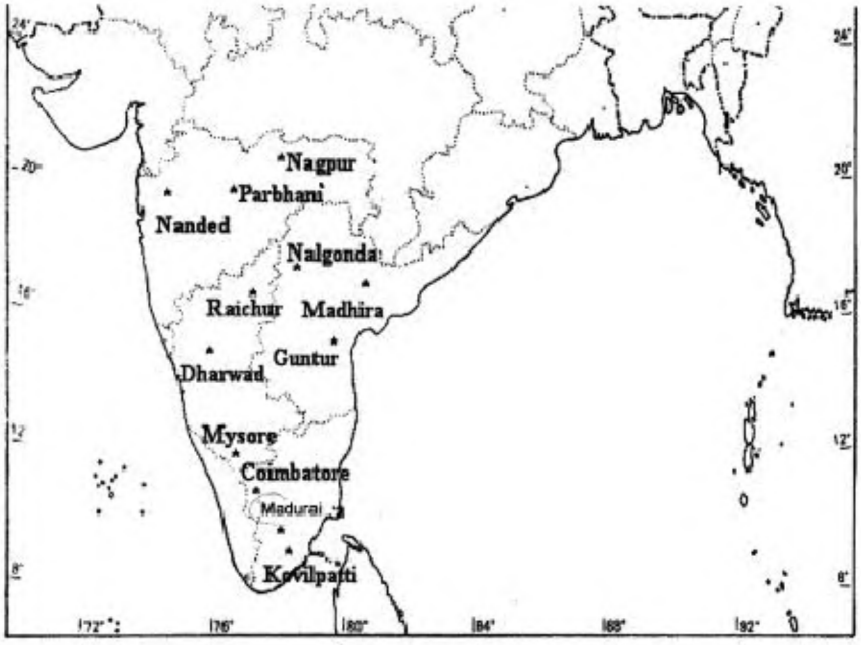

Figure 1. Geographical distribution of cotton bollworm populations in four states of South Indian cotton ecosystem used in the present investigation.

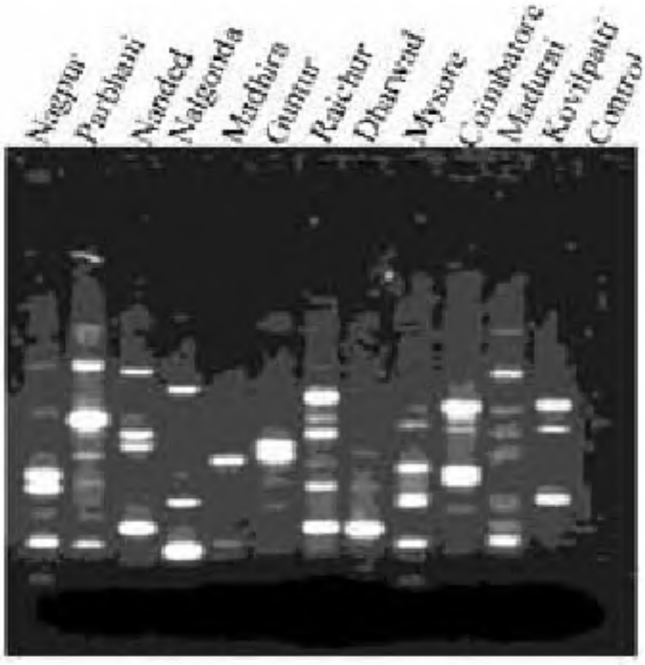

Figure 2. PCR-amplified genomic DNA of cotton bollworm using OPA 16 random primer.

individually pooled genomic DNA of all the 12 geographical populations is presented in Figure 2. A total of 497 amplicon levels resulting from 25 primers were available for analysis. The highest number of 37 amplicon levels were produced by the primer OPA 01, followed by 32 levels each by OPD 07 and OPA 05. The lowest of 7 marker levels was noticed with OPD 13 primer. On an average there were 19.88 amplicon levels per primer, of which 19.80 were polymorphic, indicating high variability among *H. armigera* populations. All the 25 primers selected for the study produced unique banding patterns that could differentiate all the 12

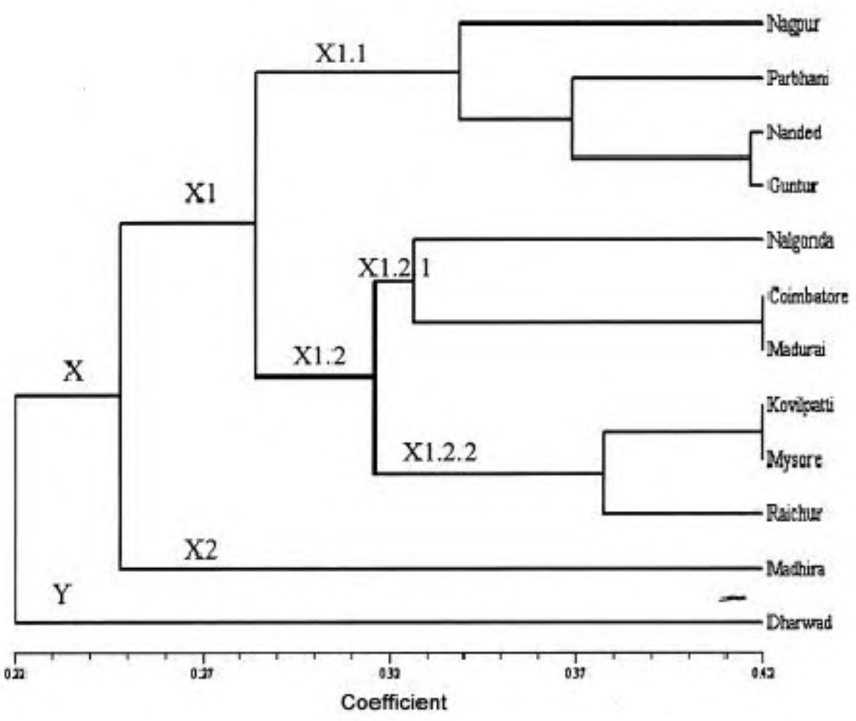

Figure 3. Dendrogram showing diversity of geographical populations of *H. armigera* in South Indian cotton ecosystem.

geographical populations. The polymorphism revealed by RAPD serves as a dominant Mendelian marker[12]. As heterozygotes are not normally detectable, results are not readily usable for computing Hardy–Weinberg gene frequencies or Nei's standard genetic distance[32]. Therefore, in the present study, RAPD polymorphisms were analysed with a phenetic distance measure (Jacquard's coefficient) from which a dendrogram was constructed, providing an indication of the diversity present within *H. armigera* in South India. Clustering analysis and principal component analysis (PCA) clearly showed two major groups, X and Y, in geographical populations of *H. armigera* (Figure 3). In PCA, the first two components accounted for 58% of the variation. The first group (X) comprised 11 populations while the distinct second group (Y) consisted of only Dharwad population. The X group is subdivided into X1 and X2 at a similarity coefficient of 0.25, where X2 comprised only one population, from Madhira. The X1 group comprising 10 geographical populations included two distinct groups (at a similarity coefficient of 0.28), X1.1 and X1.2; X1.1 group consisting of Nagpur, Parbhani, Nanded and Guntur populations is located in the northern part of South India, while X1.2 comprised populations representing Raichur, Mysore, Kovilpatti, Madurai, Coimbatore and Nalgonda. Interestingly, all these populations except Nalgonda are from the southern part of the South Indian cotton ecosystem.

It is clear that within subclusters, geographically proximate populations, viz. Coimbatore and Madurai (X1.2.1), and Kovilpatti and Mysore (X1.2.2) shared the highest similarity of about 42%. In subcluster X1.1, populations from Nanded and Guntur shared the next highest similarity. Overall, within a similarity coefficient range of 0.22 to 0.42, subclustering is by and large in agreement with the geographical proximity, except Dharwad and Madhira populations, which are distinct (Dharwad is isolated from the rest by a vast stretch of dry land having low-intensity cropping pattern with cotton/legumes). One of the important observations of the study is that none of the geographical populations of *H. armigera* studied in the South Indian cotton ecosystem shared a similarity more than 42% indicating high level of genetic differences between populations. Genetic similarities among geographical populations from the data were within the similarity coefficients ranging from 0.22 to 0.42. None of the populations compared to one another beyond a similarity coefficient equivalent to 0.42. Population similarity coefficient matrix based on shared banding pattern shows wide differences; while Madhira and Dharwad were least similar (0.15), Coimbatore, Madurai, Kovilpatti and Mysore populations were the most similar (0.42) among the lot. However, the RAPD analysis of Turkish and Israeli populations of *H. armigera* revealed low level of genetic distance suggesting high level of gene flow[33], which is contrary to the present in South Indian cotton ecosystems. Topological barriers due to weather and environmental factors and temporal barriers due to cropping pattern may play a key role in isolating some populations, resulting in high amount of genetic variability among geographical populations in South Indian cotton ecosystems. *H. armigera* being panmictic and mobile, intermating of geographic groups within the range of migration followed by isolation would result in high genetic variability within a population. The high genetic variability will help species to evolve and adapt faster to different environments; rapid evolution of resistance to insecticides, is a case in point.

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Eco-friendly way to keep away pestiferous Giant African snail, *Achatina fulica* Bowdich from nursery beds

The Andaman and Nicobar Islands in the Bay of Bengal are peaks of a submerged mountain range, arching from Myanmar to Sumatra, between latitudes 6°45′ and 13°41′N and longitudes 92°12′ and 93°57′E. The group comprises over 572 islands and rocks, with a total coastline of about 1962 km. The forest type of the Andaman and Nicobar islands can be broadly classified as tropical evergreen, with inland areas being either forest or grasslands and significant proportion of the coast being mangroves. These islands are home to 5357 species of fauna and 1454 taxa of angiosperms. Of the total species of fauna, 487 are endemic and in flora a total of 221 species are reported to be endemic to these islands.

Some of the invasive pests have invaded and established in these islands. One such pest is the Giant African snail, *Achatina fulica* (Figure 1). It was reportedly introduced during 1940s into Andamans[1]. By 1973, it was reported to have spread to a number of places in both the Andaman and Nicobar Islands[2]. During the same year it was reported that the snails were absent in the recently inhabited islands of Neil, Havelock, Little Andamans and Great Nicobar[3]. Today the snail may be seen in North, Middle, South and Little Andamans, Long Island, Car Nicobar, Katchal, Nancowry and Great Nicobar[4]. This pest is polyphagous, attacking about 225 plants of agricultural and horticultural importance, including cuttings and seedlings. Vegetables belonging to the families Cruciferae, Cucurbitaceae and Leguminoseae are known to suffer the most damage[5]. The Giant African snail is considered a serious pest of nursery beds of vegetables and flower plants. They move out of hideouts at dusk and feed throughout the night ravaging the seedlings.

Chemical control of snails typically employs metaldehyde, methiocarb (Mesurol), salt, or combinations of these chemicals with other molluscicides in a myriad of bait formulations or foliar sprays. The principal toxic effect of metaldehyde is through stimulation of the mucous glands, which cause excessive sliming, leading to death by dehydration. Metaldehyde is toxic to slugs and snails both by ingestion and absorption by the 'foot' of the mollusc. The pesticidal properties of methiocarb are similar to the toxic action of other carbamates which prevent effective nerve transmission by inhibiting the enzyme acetylcholinesterase. In addition to these molluscicides, sodium chloride – common table salt – is an effective dehydrating agent. It may be applied as a 12-inch barrier application on the perimeter of known/suspected snail-infested areas. During periods of rain or high relative humidity, salt barriers should be renewed frequently.

Various molluscicides like metaldehyde are non-selective, thus their use has a chance