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**Colour Variation and Genetic Diversity in Tea Mosquito Bug
[*Helopeltis theivora* (Hemiptera: Miridae)] Population from
Badlabeta Tea Estate, Upper Assam, India**

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Abstract: Tea mosquito bug [*Helopeltis theivora* Waterhouse (Hemiptera: Miridae)], one of the major pests of tea, have been studied in a randomly collected population sample from Badlabeta tea estate, upper Assam. DNA isolated from insects, separated on the basis of pronotum colour, show polymorphism using Randomly Amplified Polymorphic DNA primers-Polymerase Chain Reaction (RAPD-PCR) with seven primers. Phenograms on the basis of banding patterns were constructed using Numerical Taxonomy System (NTSYS-pc) version 2.02e. A similarity matrix based on the simple matching coefficient was generated by the SIMQUAL program and cluster analysis performed with the Unweighted Pair Group Method with Average (UPGMA) in the Sequential Agglomerative Hierarchical and Nonoverlapping (SAHN) program. The constructed phenogram shows that one colour variant is distinctly different from the rest three. The study indicates that the population consists of discontinuous phenotypes among individuals within a freely interbreeding population which has many of its hosts in the vicinity. Genetic variation among the phenotypes within a population focuses on some evolutionary mechanisms which may resist the effect of pesticide.

Key words: Tea mosquito bug, *Helopeltis theivora*, upper Assam, colour variation, polymorphism, RAPD-PCR

INTRODUCTION

Tea Mosquito Bug (TMB), *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) has been the major pest of tea in past as well as in recent times in North East India which causes heavy losses every year because it attacks only the young shoots which yield the actual crop of tea. Besides causing a considerable amount of crop loss in tea it also causes deterioration of quality in the prepared tea, leading to a lowering of its market value. About 80% area of the tea plantation in North East India is affected by this pest which reduces 10-50% productivity (Gurusubramanian and Bora, 2007). The average mean shoot infestation during 2005 and 2006 were 24 and 21%, respectively (Sarmah and Phukan, 2004).

Tea mosquito bug is a destructive polyphagous pest. Besides tea it also attacks cashew, guava, mango, *Jasminum scandens*, *Mikania micrantha*, *Acalypha*, *Duranta* etc. (Sundaraju and Sundarababu, 1999). In nature, polyphagous pests tend to be mono or oligophagic at the micro ecological level and their populations could be made up of individuals that are predominantly monophagous (Karowe, 1989). Hence, polyphagy at the species level does not necessarily imply polyphagy at the individual level (Cunningham *et al.*, 1999). The selective use among diverse resources may lead to the evolution of ecological specialization and adaptation (Berenbaum, 1996; Kawecki, 1997). The versatility may

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be due to the presence of a strong genetic variability making it a serious pest. In this regard a better understanding of the genetic differences of *H. theivora* can be very useful to understand the structure and population dynamics, their behavior and response to various selection pressures.

Molecular variability among 10 populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), collected from maize (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) located at distinctive geographical regions in Brazil, was assessed through RAPD-PCR (Martinelli *et al.*, 2006). One of the species of Hemiptera, *Bemisia tabaci* (Gennadius), the most important pests of agricultural crops world wide has been extensively studied. There are three biotypes-A, B and C. Molecular studies have been done thoroughly on *B. tabaci*. In Brazil, RAPD-PCR was used to survey the B biotype and other biotypes because correct identification is problematic due to highly polymorphic with extreme plasticity in key morphological characters that vary according to host (Lima *et al.*, 2000). The green bug *Schizaphis graminum* (Rondani) is one of the most important cereals pests in the world. Within populations of this species, several biotypes, which are clones that share same virulence relationships with cultivated plants, can be distinguished. Characterization and genetic relationships among Brazilian biotypes of green bug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) has been studied by using RAPD markers (Lopes-da-Silva *et al.*, 2004).

At a local scale, tea plants are usually highly homogeneous in terms of spatial distribution and quality and provide a high-level resource for consumers. Agricultural development is therefore thought to favor phytophagous insects with a high rate of increase on a plant to which they are specialized (Lombaert *et al.*, 2006).

Insect populations of the same species having diverse biological traits such as diurnal or seasonal activity patterns, size, shape, color, insecticide resistance, migration and dispersal tendencies, pheromone differences, or disease vector capacities have been designated as biotypes. Biotypes occur due to nongenetic polyphenisms or genetic polymorphisms or geographical isolation (Diehl and Bush, 1984). The heavy use of pesticides, intensive production and an insect pest species with the biotic potential to exploit a wide resource base within a permissive environment may have contributed to the aggregation of strains or races which are genetically different from each other (Wool *et al.*, 1993; Toscano *et al.*, 1998).

The present study aims to find out phenotypic and genetic variation within a population of *H. theivora* from Badlabeta Tea Estate, upper Assam.

MATERIALS AND METHODS

Tea mosquito bugs were collected randomly from Badlabeta Tea Estate (Tinsukia District), Assam during April to October, 2008-2009. The collected samples separated on the basis of colour variation of the pronotum and photographed in zoom stereomicroscope (Meiji EMZ-TR, Meiji Techno Co, Ltd, Tokyo, Japan) in the laboratory of Tocklai Experimental Station. DNA was extracted from different variants based on colour following standard protocol (Barro and Driver, 1997) with minor modifications. PCR amplifications were performed using a set of seven RAPD primers (OPA-02, OPA-08, OPA-11, OPD-05, OPD-08, OPD-12 and OPD-20) from Operon Technologies, Inc., Alamada, Calif. Amplifications were carried out in Applied Biosystem 2720 Thermal Cycler with an initial denaturation of 180 sec at 94°C followed by 45 cycles consisting of 60 sec denaturation at 93°C, 60 sec annealing at 35°C and 120 sec extension at 72°C with a final extension of 300 sec at 72°C. To reduce the possibility of variation in amplification reaction, master mixing of reaction constituents was always used. PCR reaction mixture of 25 µL comprised of 1x buffer, 0.2 mM dATP, dCTP, dGTP, dTTP, 2 mM MgCl₂, 0.2 µM of primer, 50 ng of template DNA and 0.25 U of Taq DNA polymerase. Amplified products were electrophoresed in 1.8% agarose gel with 100 bp DNA ladder (Genei) as size marker.

Phenogram based on the banding patterns were constructed using the Numerical Taxonomy System (NTSYS-pc) version 2.02e (Rohlf, 1998). Only the major bands were considered. The presence of the band was coded as 1 and its absence as 0. A similarity matrix based on the simple matching coefficient was generated by the SIMQUAL program and cluster analysis performed with the Unweighted Pair group method with average (UPGMA) in the SAHN (Sequential agglomerative hierarchal and nonoverlapping) program (Rohlf, 1998).

Polymorphic Information Content (PIC) values were calculated for each RAPD primer according to the formula:

$$PIC = 1 - \sum (P_{ij})^2$$

where, P_{ij} is the frequency of the i th pattern revealed by the j th primer summed across all patterns revealed by the primers (Botstein *et al.*, 1980).

RESULTS

In the collected samples the female population (Fig. 1A-D) shows variation in the colour of the pronotum. The colors of the pronotum are yellow (Fig. 1A), reddish brown (Fig. 1B), yellowish brown (Fig. 1C) and light brown (Fig. 1D). The distribution of colour variants in the female population shows that percentage of yellowish brown is maximum and percentage of reddish brown with black spots is minimum (Fig. 2).

PCR amplifications with random primers show polymorphisms among the female colour variants (Fig. 3A-F). Amongst the random primers OPD-12 show maximum PIC value where as OPA-02 minimum PIC value whereas percent polymorphism is highest in OPD-05 and lowest in OPD-12 (Table 1).

Of the four colour variants under study, one colour variant (reddish brown) could be differentiated from the rest three as it diverged early, at similarity coefficient of 0.38 as revealed from



Fig. 1: Colour variation in female population of *H. theivora* showing (A) yellow (X 20), (B) reddish brown (X 20), (C) yellowish brown (X 20) and (D) light brown (X 20)

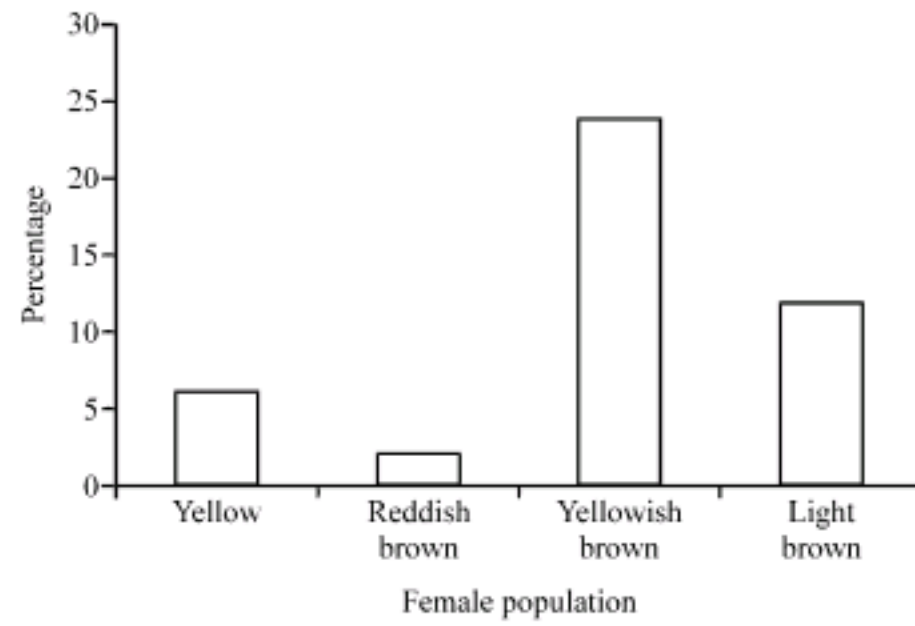


Fig. 2: Distribution of colour variation of female *H. theivora* within a population

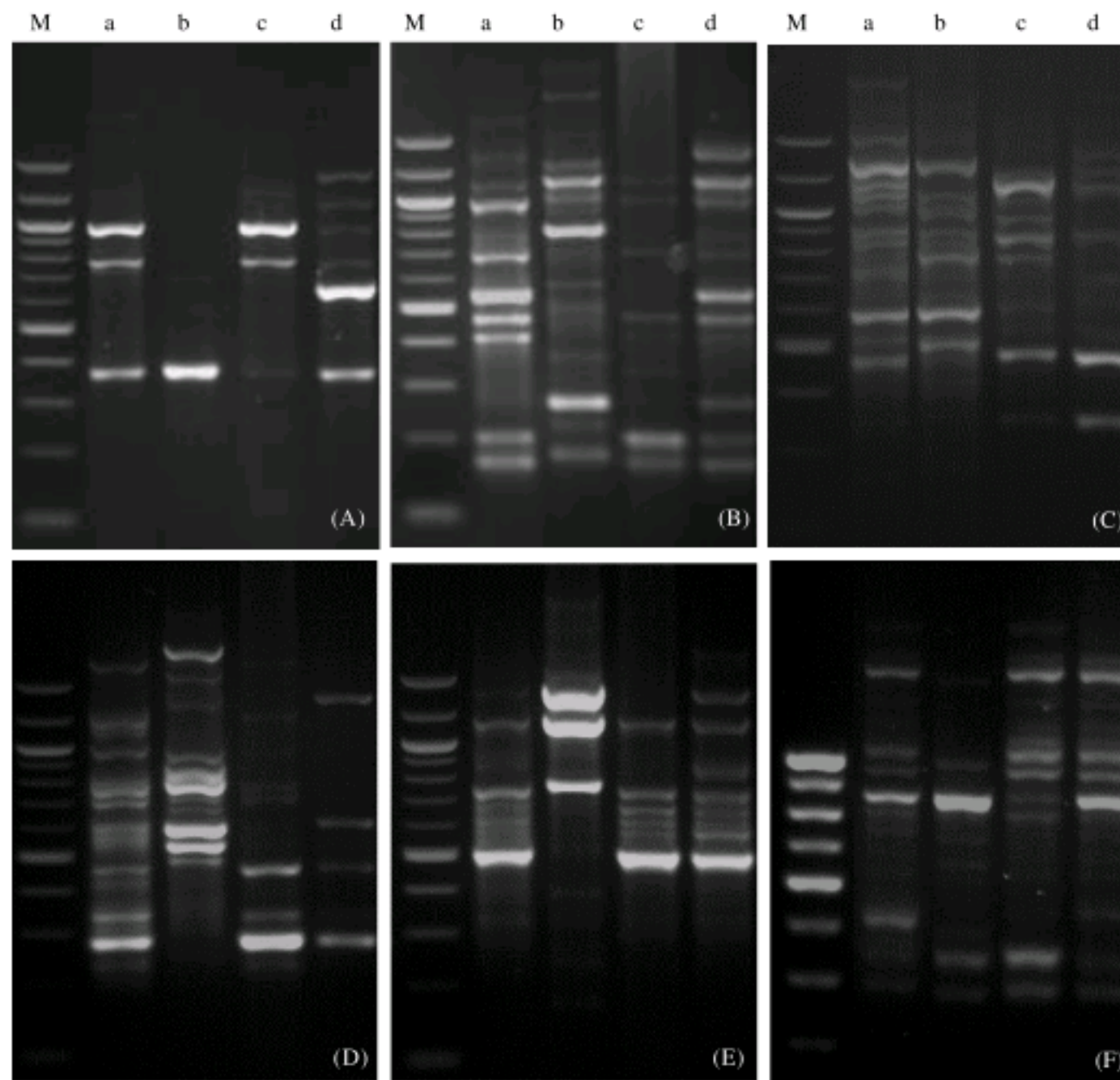


Fig. 3: RAPD profiles of colour variants of *H. theivora* with (A) OPA 02, (B) OPA 08, (C) OPA 11, (D) OPD 05, (E) OPD 08 and (F) OPD 12 primers: Lane M: 100 bp ladder (Genei); Lane a: Yellow; Lane b: Reddish brown; Lane c: Yellowish brown and Lane d: Light brown

the phenogram constructed (Fig. 4). The three colour variants (yellow, yellowish brown and light brown) further split at similarity coefficient of 0.70, again leaving two in one group. The rest two colour variants (yellow and yellowish brown) again split at 0.71.

Table 1: Percent polymorphism and polymorphic information content of RAPD primers used in PCR

Primers	Sequence	Percent polymorphism	PIC value
OPA-02	5'-TGCCGAGCTG-3'	88.89	0.177
OPA-08	5'-GTGACGTAGG-3'	87.50	0.203
OPA-11	5'-CAATCGCCGT-3'	81.25	0.188
OPD-05	5'-TGAGCGGACA-3'	100.00	0.269
OPD-08	5'-GTGTGCCCA-3'	86.67	0.267
OPD-12	5'-CACCGTATCC-3'	76.47	0.346
OPD-20	5'-ACCCGGTCAC-3'	69.23	0.192

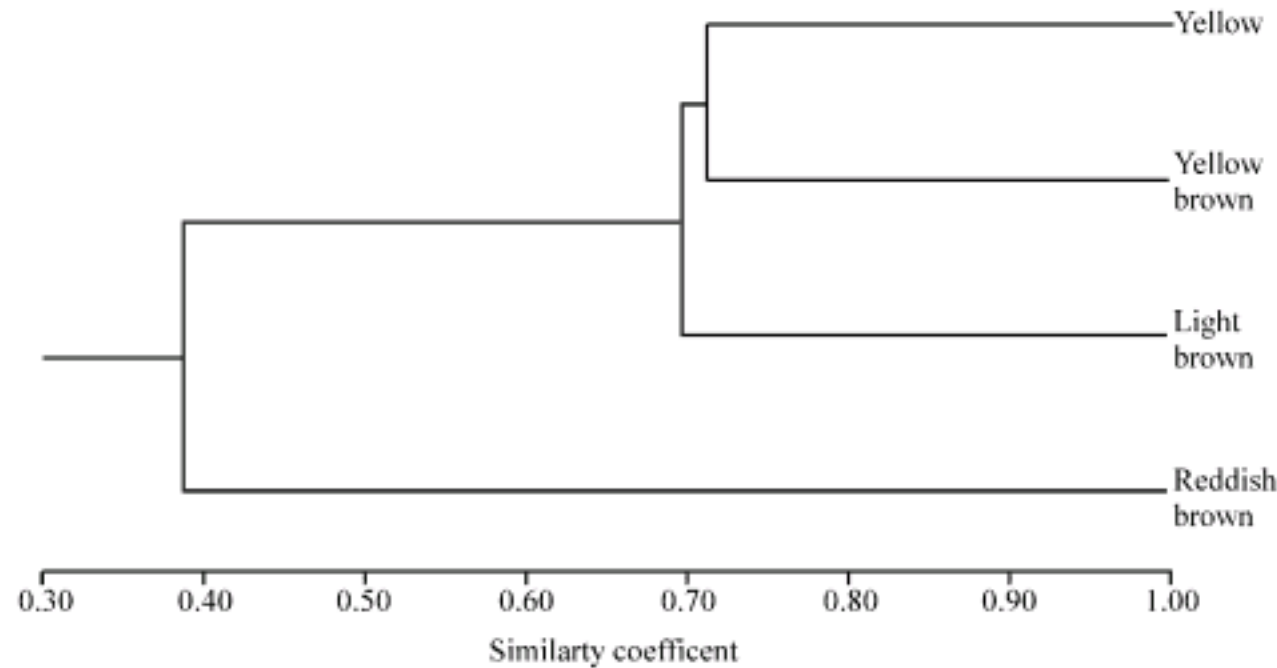


Fig. 4: Phenogram of four *Helopeltis theivora* colour variants based on polymorphic bands resulting from PCR mediated RAPD analysis

DISCUSSION

The genetic polymorphisms detected using RAPD-PCR among colour variants show that *Helopeltis theivora* population consists of discontinuous phenotypes among individuals within a freely interbreeding population. The colour variants within *H. theivora* population may be considered as different forms of same species i.e., biotypes. Genetic variation among biotypes may focus on some evolutionary mechanisms (Futuyma and Peterson, 1985) to resist the effect of pesticides.

The study is important in an ecosystem where a polyphagous insect like *H. theivora* has many of its hosts in the vicinity which may lead to interbreeding between isolated populations. Such an interbreeding phenomenon between varying host associated populations indicates the presence of natural refugia in alternate hosts. However, the degree of polyphagy expressed by individual *H. theivora* in the field is still unclear. Egg laying females could utilize a number of hosts or restrict laying to a single host.

The host-races and biotypes within pest populations are sometimes obstacles to pest management. Detailed field level investigations on the polyphagy of individual *H. theivora* and the mating behavior of such individual populations combined with evaluation of their genetic diversity and pesticide resistance remains to be done.

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