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Assessment of dispersal patterns of harvester termite *Anacanthotermes viarum* (Konig) using RAPD analysis

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Abstract

Dispersal patterns of harvester termite *Anacanthotermes viarum* (Konig) was analysed using RAPD of DNA sequences obtained from eleven populations from southern Tamil Nadu, South India. Two dispersal patterns *viz.,* the Vaigai tract with the populations from Varushanadu, Chitalangudi, Madurai, Valayankulam, Paramakudi and Ramanathapuram and the dry tract of Southern Tamil Nadu with the populations of Vijayanarayanam, Nainarpuram, Kalligudi, Kovilpatti and Andipatti) could be discerned from genetic similarities. The termites from Varushanadu were unique and seemed to have moved from Southern tract and Vaigai tract. This movement of harvester termites from other parts to Varushanadu might be due to the impact of climatic conditions.

Keywords : *Anacanthotermes viarum*, dispersal patterns, harvester termite, PCR analysis, RAPD

INTRODUCTION

Anacanthotermes viarum (Konig) is distributed in and around dry tracts of Rajasthan, Pakistan and drier parts of southern India (Chhotani and Bose, 1973). It was documented on grass by Roonwal (1970). It damages the rice crop by cutting the leaves and takes the cut bits to the underground nest. Ravi (1992) first reported this species as a pest of upland rice in Ramanathapuram district and opined that they are prevalent only in dry regions of Ramanathapuram and Tirunelveli districts. Similar findings were reported by Nelson *et al*. (1994) and Rajavel and Venugopal (2001). Though it was commonly known as a species of drier parts, Bose (1984) reported *A. viarum* from hill area (*Idumbamalai*). Thus, the threat of *A. viarum* has been getting extended from drier parts to hill areas as well.

Comprehensive knowledge of the genetic structure of termite populations could provide insight into social and spatial organization as well as dispersal patterns of colonies and thus could facilitate remedial and regulatory control efforts (Hussender *et al.* (2002). Indeed Jenkins *et al.,* (2002) used the DNA sequencing data of *Coptotermes formosanus* Shiraki to assess the impact of interstate commerce and the likely origin of Formosan subterranean termite infestation in Atlanta, Georgia. Similarly, the molecular characterization of eighteen new populations of *Reticulitermes* was studied by Luchetti *et al*. (2004) and found the northeastern Italian *Reticulitermes sp* which was more widely distributed in northern and south eastern Italy to show a close relationship to the sample from Peloponnese, while the gene bank sample from continental Greece, on the contrary appeared more related to other eastern taxa such as *R. lucifugus* from Turkey and *R. clypeatus* from Israel. Their study also evidenced instances of anthropogenic involvement in taxa distribution of these termites.

The present study on the DNA sequence data of harvester termites of southern Tamil Nadu, India was carried out to find out the implications of dispersal of *A. viarum* from drier parts to hill region.

MATERIALS AND METHODS

Location of harvester termite collection sites

The harvester termites were collected along the course of River Vaigai (Southern Tamil Nadu, India. Fig 1.) *viz.,* Varushanadu (Theni district), Andipatti (Theni district), Chittalangudi (Madurai district), Vaigai river bank (Madurai district), Paramakudi (Ramanathapuram district) and Ramanathapuram (Ramanathapuram district). They were also collected from the following dry places: Valayankulam and Kalligudi (Madurai district), Kovilpatti (Tuticorin district) and Nainarpuram and Vijayanarayanam (Tirunelveli district). The study area covers 8^{o} to10^{o} N and 77^{o} W to 79^{o}E of southern Tamil Nadu, India.

DNA isolation

Forty mg muscle tissue of the workers of the harvester termite *A. viarum* was ground with 300 micro litre of CTAB (cetyl trimethyl ammonium bromide) DNA extraction buffer (1% W/V CTAB; 1.4M NaCl; 10mM EDTA, pH 8.0; 100mM Tris-HCl, pH8.0; 0.2% V/V b mercaptoethanol). The mixture was emulsified with equal volume of phenol: chloroform (1:1). It was centrifuged at 10,000 rpm for 5 min at room temperature. The aqueous phase was collected and mixed with equal

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Figure 1. Distribution patterns of *Anacanthotermes viarum* (Konig) in Southern Tamil Nadu, India

volume of chloroform: isoamyl alcohol (24:1). The mixture was then centrifuged at 10,000 rpm for 5 min and the ethanol was air dried. The pellet was dissolved in 50 ml of TE buffer (Tria 10mM, pH 8.0 and EDTA 1mM, pH 8.0). The isolated DNA was quantified by spectrophotometer (260nm) and quality was tested by agarose gel electrophoresis.

RAPD-PCR Analysis

The DNA (20 ng) was dissolved in 20ml PCR reaction buffer containing 10mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.2 mM dNTPs, 21 pM of primer and 0.5 U of DNA polymerase. The primer RAPD Kit A15 obtained from IDT was used for RAPD-PCR studies. PCR was conducted according to the methods of Williams *et al.*, (1990) which includes various steps like, initial heat step (94°C for 5 min.), 40 cycles of denaturation (94°C for 1 min.), annealing (36°C for 1 min.) and extension (72°C for 2 min.) and a final extension step (72°C for 7 min.). Amplification was performed using a programmable thermal cycler PTC-150 (MJ Research, USA). The products of PCR and DNA size markers [l DNA digested with EcoRI and Hind III (Bangalore Genei)] were loaded onto a 1.6% tris-borate-EDTA agarose gel and run for 4hrs at 50V. The gels were stained with ethidium bromide and photographed. Each lane of RAPD profiles was subjected to gel documentation system (Vilbert-Lourmat, France). The dendrogram analysis was carried out using BIO-Profile 1D software (LTF-Labortechnik, Nasserburg, Germany) (Vilbert Lourmat, France).

RESULTS AND DISCUSSION

The genetic variability of *A. viarum* showed that there was a similarity as well as variation between the populations from different places. RAPD DNA profile (Fig. 2.) revealed that the populations from Varushanadu, Chitalangudi, Madurai, Valayankulam, Paramakudi and Ramanathapuram were similar with 1,312 base pairs of DNA. Vijayanarayanam, Nainarpuram, Kalligudi, Kovilpatti and Andipatti populations had similar base pair of 1904. Varushanadu population was unique in having both the base pairs of 1312 and 1904. This indicated that the population of Varushanadu might have come from eastern and southern parts of Varushandu *ie* from Vaigai tract and southern tract. Since these parts are dry tracts the population might have moved to Varushanadu (600 MSL) which is a hilly area and having crops and grasses through out the year (Fig 1).

Average homology coefficient per cent was 85 for Valayankulam population with Chittalangudi, Ramanathapuram, Paramakudi, Madurai and Varushanadu (Table 1 and Fig. 3). This might be due to dispersal from Ramanathapuram to Varushanadu along Vaigai. Though the populations from Varushanadu shares unique base pairs of DNA with populations of Vijayanarayanam, Nainarpuram, Kalligudi, Kovilpatti and Andipatti, it has an average of 22% homology co efficient with these populations. Unique observations were Andipatti and Nainarpuram populations which shared 100% similarity with each other. The Andipatti population may be considered as a population, which came from Nainarpuram. Thus the climatic changes and availability of vegetation seemed to have played a major role in the distribution of termite populations in the present study area. This was in accordance with Ward (1987) who studied the dispersal pattern of argentine ant *Iridomyrmex bumilis* in the riparian

Table 1. Similarity Index (Homology coefficient %) based on RAPD Profiles of termite (*A. viarum*) populations of Southern Tamil Nadu, India (1 - Vijayanarayanam; 2-Nainarpuram; 3-Kalligudi; 4-Kovilpatti; 5-Andipatti; 6-Varushanadu; 7-Madurai; 8-Paramakudi; 9-Ramanathapuram; 10-Chittalangudi; 11-Valayankulam)

	1	2	3	4	5	6	7	8	9	10	11
1	1.00										
2	0.50	1.00									
3	0.75	0.75	1.00								
4	0.25	0.75	0.50	1.00							
5	0.25	1.00	0.75	0.75	1.00						
6	0.44	0.22	0.22	0.22	0.22	1.00					
7	0.67	0.22	0.22	0.22	0.22	0.80	1.00				
8	0.67	0.44	0.22	0.22	0.22	0.80	1.00	1.00			
9	0.44	0.22	0.22	0.22	0.22	0.80	0.80	0.80	1.00		
10	0.44	0.22	0.22	0.22	0.22	0.80	1.00	0.80	0.80	1.00	
11	0.67	0.44	0.22	0.22	0.22	0.80	1.00	1.00	0.80	0.80	1.00

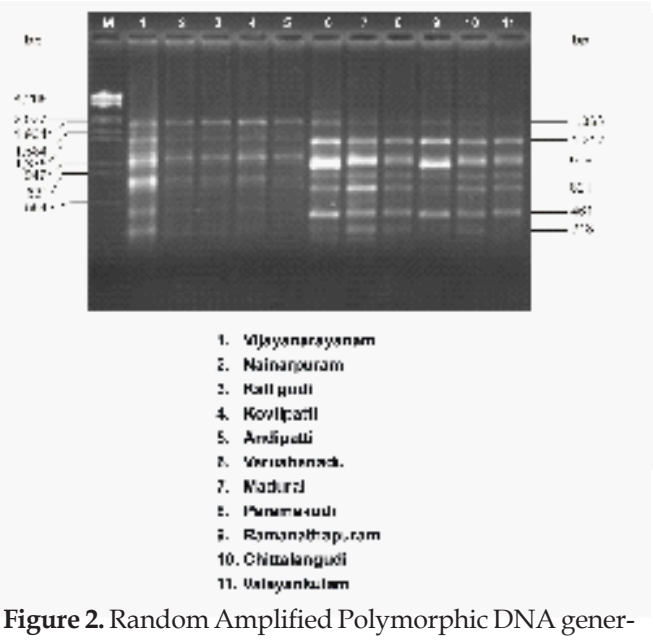

Figure 2. Random Amplified Polymorphic DNA generated for the termite (*A. viarum*) populations of Southern Tamil Nadu, India

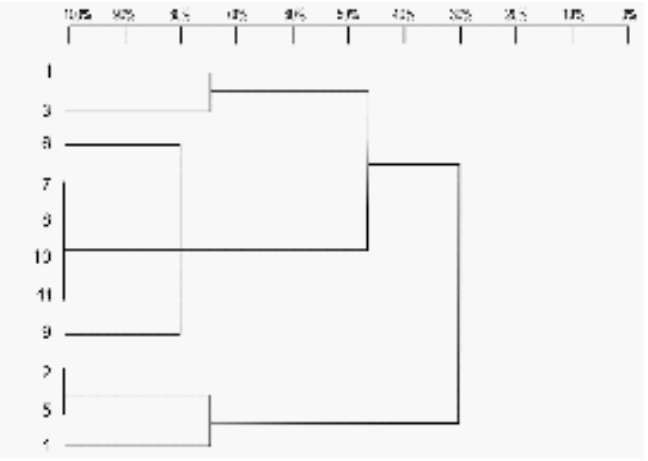

Figure 3. Dendrogram using Homology Co efficient (%) to show the similarities in RAPD profiles of termite (*A. viarum*) population of Southern Tamil Nadu, India (1 - Vijayanarayanam; 2-Nainarpuram; 3-Kalligudi; 4-Kovilpatti; 5-Andipatti; 6-Varushanadu; 7-Madurai; 8-Paramakudi; 9-Ramanathapuram; 10-Chittalangudi; 11-Valayankulam)

woodland of the Sacramento valley floor Yolo and Solano counties where the species invaded disturbed chaparral, coastal scrub, and other nonriparian habitats in the San Francisco Bay region with cooler and more humid climate and absent from the chaparral and oak-pine woodland with extreme summer aridity.

Thus the present study demonstrated the applicability of DNA sequencing for tracing the origin of termite infestations, which when used in conjunction with traditional or historic sources can provide insight into population structure and information on the dispersal of an introduced termite pest (Jenkins *et al*., 2002). Further, such information from DNA analysis of harvester termites will help to facilitate development of novel treatment strategies and a better understanding of the social and ecological considerations to prevent the transport and establishment of this insect pests.

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