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Genetic variability of the human filarial parasite, *Wuchereria bancrofti* in South India

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Abstract

The genetic variability of the lymphatic filarial parasite *Wuchereria bancrofti*, from three localities (one urban and two rural areas) in southern India, endemic for filariasis was studied using random amplified polymorphic DNA (RAPD) markers. The RAPD profiles were generated for 21 parasite populations (7 populations from each area), using a 10-mer random primer. The analysis of profiles indicated the existence of considerable genetic variability among parasite populations. The Nei's gene diversity between the individual populations in the 2 areas (one urban and another rural) was comparatively greater (0.3372 ± 0.1462 & 0.2830 ± 0.1764) than that of populations in another village (0.0490 ± 0.1373). The greater genetic diversity among the former areas may be due to human migration, endemicity for long time and drug (diethyl-carbamazine citrate) pressure unlike the populations of latter village where the filariasis is relatively a recent introduction and which was never under active chemotherapy. The Nei's genetic distance was estimated and the phylogenetic tree was constructed using 'UPGMA'. These analyses indicated the prevalence of at least two genetically distinct clusters, among the populations studied, their maximum genetic distance being 0.2444. The finding of two genetic 'variants' of *W. bancrofti*, in the present study, may have important implications in filariasis epidemiology and control/elimination programmes. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Genetic variability; *Wuchereria bancrofti*; Lymphatic filariasis; RAPD analysis

1. Introduction

Lymphatic filariasis is a debilitating scourge of the people living in the tropical countries of the world, affecting about 120 million people (WHO, 1998). The nematode parasite, *Wuchereria bancrofti* is the major cause of this disease accounting

for 90% of the cases. India contributes to about 40% of cases of Bancroftian filariasis in the global scenario (Ramaiah et al., 2000). The existence of three physiological strains of this parasite, based on the periodicity of appearance of microfilaria (mf) in the peripheral blood of the human host, viz., nocturnally periodic, nocturnally sub-periodic and diurnally sub-periodic (Sasa, 1976), has been reported. Also, ecological races (Sasa, 1976) based on the vector species involved in the transmission of the parasite have been described. Two

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variants of the species, on the basis of the length of mf have been observed (Chatterjee, 2001—personal communication). In India, while only the nocturnally periodic strain of the parasite occurs in the mainland, both diurnally periodic and nocturnally periodic strains are reported from Andaman and Nicobar islands (Tewari, et al., 1995; Shriram et al., 1996). The major ecological races prevalent in India are (i) *Culex quinquefasciatus* type (the major type throughout mainland), (ii) Anopheles type (*An. philippinensis* acts as the vector and has very limited distribution in the northeastern part of the country) and (iii) Aedes type (Andaman Nicobar Islands) (Sasa, 1976; Tewari et al., 1995). Also, considerable and widespread variability in the disease pattern and their response to chemotherapy has been reported for *W. bancrofti* (Sasa, 1976; Rao et al., 1977).

Recently, a global chemotherapeutic programme to eliminate lymphatic filariasis by mass drug treatment has been launched. In view of the large-scale disease control operations using chemotherapy strategies, population genetic studies are important, as the drug pressure is likely to lead to genetic variability among parasite populations. This could be due to selection for resistant strains, resulting in an increase in their frequency. The susceptible 'refugia' parasite population, present in vectors or untreated mf carriers, could neutralize this selection for resistance alleles. (Anderson et al., 1998). Ultimately this may lead to the fixation of variants and differentiation between populations. Therefore, a study was carried out to understand the genetic variability and evolutionary relationship of *W. bancrofti* populations from three localities in southern India. RAPD (random amplified polymorphic DNA) genetic marker, a useful tool for analysing the inter- and intra-specific genetic variations and phylogenetic relationship (Williams et al., 1990; Singh, 1997; Gomes et al., 2000; Tcherneva et al., 2000) of anonymous genomes (Hadrys et al., 1992), was used for the study.

For a parasite like *W. bancrofti*, which is being introduced into the human system by infective vector mosquitoes, a single patent infection is a result of multiple infective bites (Rajagopalan et al., 1977). Hence, a single mf carrier represents

many lineages of parasites and therefore the intra-population variability of the parasites in a human carrier. This could be a reflection of the parasite populations in the transmissible vicinity of the human host. In the present study, the parasites (microfilariae) collected from a single individual (mf carrier) constituted a parasite population. The parasite populations collected from different individuals of a village constituted inter individual population level and those from different villages/towns formed inter group population level (groups). These groups of populations were selected in such a way that, the exposure of these populations to operational chemotherapeutical measures were kept a variant, so as to have an understanding of the genetic variability as a resultant of exposure to chemotherapeutical operations.

2. Materials and methods

2.1. Study area and collection of samples

Blood samples were collected from microfilaraemic individuals living in Pondicherry town, (Pondicherry union territory, India), Chinnanergunam and Athipakkam villages (located in Tindivanam and Thirukoilur 'taluks' respectively in Villupuram District of Tamil Nadu State, India) (Fig. 1). These places are located at a distance of about 40 km from each other. Pondicherry, an urban agglomeration with a population of 401 437 (Census report, Govt of India, 1991) has been endemic for Bancroftian filariasis for decades (Das et al., 1992) and selective diethyl-carbamazine citrate (DEC) therapy and vector control measures have been in operation at least for the last half a century. Chinnanergunam (Population: 1921) is an endemic village (Ramaiah et al., 1996) and DEC therapy has been operational for at least the last 10 years. Athipakkam village (Population: 2547) had never been under active DEC therapy and was found recently to be endemic for filariasis.

Five milliliters of venous blood were collected from seven microfilaraemic carriers (mf density per 20 mm³ ranged from 10 to 331) from each

Table 1
Standardization of RAPD primer sequences for the study

Primers	Number of fragments amplified					
	Area 1		Area 2		Area 3	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
1	14	6	10	13	9	12
2	7	0	0	5	6	3
3	0	0	0	0	0	0
4	1	0	7	10	2	7
5	7	0	7	1	2	9
6	0	0	0	0	0	0

run on a 1.0% agarose gel along with λ /Hind III + ϕ X 174/Hae III, ladder, the gel was stained with ethidium bromide and photographed using Polaroid film. The size of the bands was determined by TotalLab v1.10 software (Phoretics, USA). They were normalized and poorly visible bands were not taken into consideration for analysis. RAPD analysis of human DNA (uninfected), using the same primer following similar protocol was also conducted to rule out the possibility of contamination of human DNA in the samples. Also, a negative control (with no DNA) was run always when a RAPD analysis was done. The amplification of all the DNA samples was repeated three times in order to see variability, if any, in the amplification pattern.

2.3. Gene diversity and phylogenetic analysis of the RAPD profile

The Nei's gene diversity (Nei, 1978) among the individual populations (parasites from one patient) of *W. bancrofti* from each area was estimated so as to have an understanding of the heterogeneity in the population in an area. Unweighted Pair Group Method with Arithmetic mean (UPGMA) of the RAPD profile was carried out to analyse the phylogenetic relationships between the individual populations and group of populations (the populations sampled from an area was grouped together) between different areas. This analysis was done after plotting Nei's

unbiased genetic distance matrix (Nei, 1978), using 'PopGene 32' software (Francis et al., 1999).

3. Results

3.1. Selection of a suitable primer

In order to identify a suitable primer for the study, six primers (RAPD primer kit, Amersham Pharmacia Biotech, Sweden) were used to amplify two parasite DNA samples from each of the three areas. Primer 1 (5' d[GGTGCGGGAA] 3') amplified all the samples (Table 1) yielding 6–14 DNA fragments of different sizes (VCRC, 1999) and was used for further studies.

The RAPD profile was generated for 21 *W. bancrofti* populations from three different areas (Fig. 2). A total of 40 fragments were amplified from all the populations, which ranged in size from 468 to 2348 bp. However, the number of DNA fragments amplified for each sample ranged from 7–18. Only one fragment (1003 bp) was found to be common for all the 21 populations studied. The uninfected blood sample processed parallelly did not show amplification of any fragment. Amplification of human DNA (non-infected with *W. bancrofti*) with the same primer under similar conditions yielded 12 fragments ranging from 389 to 2048 bp and none of these fragments was identical to the 40 fragments amplified in the *W. bancrofti* DNA samples (Table

2). Both these observations show that there was no human DNA contamination in the parasite DNA samples used for this study.

The analysis of the RAPD profiles showed that the Nei's gene diversity (Nei, 1978) among the individual populations from Pondicherry and Chinnanergunam was found to be comparatively greater (0.3372 ± 0.1462 & 0.2830 ± 0.1764 respectively) than that of Athipakkam (0.0490 ± 0.1373). This reveals a high degree of genetic variability between individual populations from the former two localities compared to that of the populations in the latter village.

The unbiased Nei's genetic distance (Nei, 1978) matrix computed for individual populations of *W. bancrofti* is presented in Table 3. The genetic distance between the individual populations ranged from 0 to 0.7985. The analysis of the genetic distance yielded a phylogenetic tree, which exhibited two distinct clusters. One of the clusters

included all the populations from Chinnanergunam while the other had the populations of Pondicherry and Athipakkam. The populations of Athipakkam village exhibited a close relatedness (Fig. 3).

When the populations of the three localities were analyzed as groups, (PopGene 32) a genetic distance of 0.2444 was obtained between Chinnanergunam and Athipakkam populations of *W. bancrofti*, while that between Chinnanergunam and Pondicherry was 0.1632 and between Athipakkam and Pondicherry was only 0.1572. The Phylogenetic tree generated by group analysis revealed a genetic relationship similar to that obtained for individual populations (Fig. 4).

4. Discussion

The results of the present study, using RAPD genetic markers, indicated the existence of considerable genetic variability of *W. bancrofti* parasite populations. Such variabilities have been reported for other human parasitic nematodes, *Ascaris* (Anderson, et al., 1998), *Trichinella spiralis* (Sequeira et al., 2000), *Onchocerca volvulus* (Anderson et al., 1998) and *Trichuris trichiura* (Currie et al., 1998) also, employing genetic markers viz., RAPD analysis, mtDNA-RFLP, isoenzymes etc.

The gene diversity was high among individual populations of an urban area and a rural locality. This may be due to the gene flow effected by the migration in the human population. Pondicherry is an urban agglomeration characterized by the influx of human population from surrounding endemic districts and states for trade and employment opportunities. This may result in influx of infected population (and thereby the parasite) from different areas. Epidemiology of filariasis is complex and the parasites undergo development in a vector phase (*Culex quinquefasciatus* acts as the vector species of nocturnally periodic *W. bancrofti* prevalent in South India) and human phase. Further, a patent filarial infection is a result of multiple infective bites to human population (Rajagopalan et al., 1977). Hence, influx of infected population would result in a mixing up of genetically different populations and thereby a greater

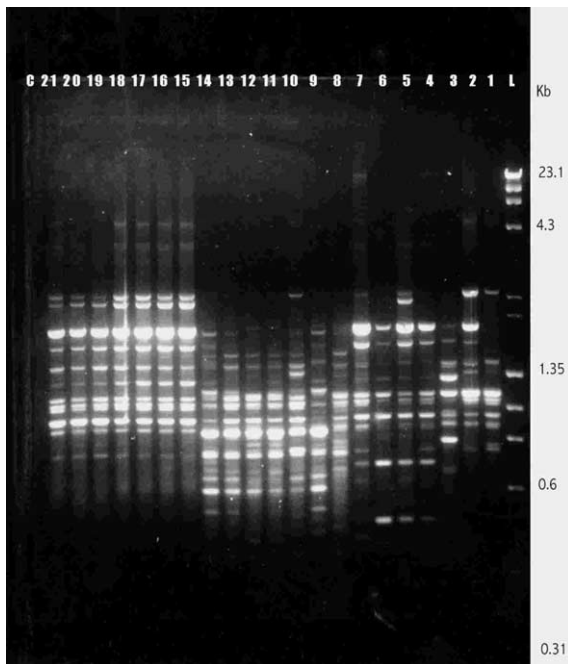


Fig. 2. RAPD profile of different samples of *W. bancrofti* with primer 1. Agarose gel electrophoresis of RAPD-PCR product: Lane 1–7 (Pondicherry), Lane 8–14 (Chinnanergunam), Lane 15–21 (Athipakkam), L (Lambda/Hind III—Phi X 174/Hae III fragments) and C—Negative Control (uninfected blood sample).

Table 3
 Nei's unbiased measures of genetic identity and genetic distance of populations of *W. bancrofti* from different regions of South India

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	****	0.7750	0.7250	0.8000	0.7500	0.6750	0.8000	0.6500	0.5750	0.7250	0.7000	0.7250	0.7500	0.6000	0.8000	0.8000	0.7750	0.8000	0.7750	0.7750	0.7500
2	0.2549	****	0.7000	0.8250	0.8250	0.8000	0.8750	0.5750	0.6500	0.7000	0.6250	0.6000	0.6750	0.5750	0.8250	0.8250	0.8500	0.8250	0.8500	0.8500	0.8750
3	0.3216	0.3567	****	0.6750	0.6750	0.7500	0.6750	0.4750	0.6000	0.5500	0.6250	0.5500	0.6250	0.6250	0.6750	0.6750	0.6500	0.6750	0.6500	0.6500	0.6250
4	0.2231	0.1924	0.3930	****	0.8500	0.8750	0.8000	0.6000	0.6250	0.6750	0.6500	0.6750	0.7500	0.6000	0.8000	0.8000	0.8250	0.8000	0.8250	0.8250	0.8000
5	0.2877	0.1924	0.3930	0.1625	****	0.8250	0.8500	0.4500	0.5250	0.5750	0.5500	0.5250	0.6000	0.5000	0.8000	0.8000	0.8250	0.8000	0.8250	0.8250	0.8000
6	0.3930	0.2231	0.2877	0.1335	0.1924	****	0.7250	0.4750	0.6000	0.5500	0.6250	0.5500	0.6250	0.5750	0.7250	0.7250	0.7500	0.7250	0.7500	0.7500	0.7250
7	0.2231	0.1335	0.3930	0.2231	0.1625	0.3216	****	0.5500	0.5750	0.6750	0.6000	0.5750	0.6500	0.6500	0.8000	0.8000	0.7750	0.8000	0.7750	0.7750	0.8000
8	0.4308	0.5534	0.7444	0.5108	0.7985	0.7444	0.5978	****	0.5750	0.6750	0.7000	0.7750	0.7500	0.6500	0.6500	0.6500	0.6250	0.6500	0.6250	0.6250	0.6500
9	0.5534	0.4308	0.5108	0.4700	0.6444	0.5108	0.5534	0.5534	****	0.7000	0.6750	0.6500	0.7750	0.8750	0.5750	0.5750	0.6000	0.5750	0.6000	0.6000	0.5750
10	0.3216	0.3567	0.5978	0.3930	0.5534	0.5978	0.3930	0.3930	0.3567	****	0.7250	0.7500	0.8750	0.8000	0.7250	0.7250	0.7000	0.7250	0.7000	0.7250	0.7250
11	0.3567	0.4700	0.4700	0.4308	0.5978	0.4700	0.5108	0.3567	0.3930	0.3216	****	0.8750	0.8000	0.8000	0.7500	0.7500	0.7250	0.7500	0.7250	0.7250	0.7000
12	0.3216	0.5108	0.5978	0.3930	0.6444	0.5978	0.5534	0.2549	0.4308	0.2877	0.1335	****	0.8250	0.7250	0.7250	0.7250	0.7000	0.7250	0.7000	0.7000	0.6750
13	0.2877	0.3930	0.4700	0.2877	0.5108	0.4700	0.4308	0.2877	0.2549	0.1335	0.2231	0.1924	****	0.1625	0.7500	0.7500	0.7250	0.7250	0.7000	0.7250	0.7000
14	0.5108	0.5534	0.4700	0.5108	0.6931	0.5534	0.5978	0.4308	0.1335	0.2549	0.2231	0.3216	0.1924	****	0.6500	0.6500	0.6250	0.6500	0.6250	0.6250	0.6000
15	0.2231	0.1924	0.3930	0.2231	0.2231	0.3216	0.2231	0.4308	0.5534	0.3216	0.2877	0.3216	0.2877	0.4308	****	1.0000	0.9750	1.0000	0.9750	0.9750	0.9500
16	0.2231	0.1924	0.3930	0.2231	0.2231	0.3216	0.2231	0.4308	0.5534	0.3216	0.2877	0.3216	0.2877	0.4308	0.0000	****	0.9750	1.0000	0.9750	0.9750	0.9500
17	0.2549	0.1625	0.4308	0.1924	0.1924	0.2877	0.2549	0.4700	0.5108	0.3567	0.3216	0.3567	0.3216	0.4700	0.0253	0.0253	****	0.9750	1.0000	1.0000	0.9750
18	0.2231	0.1924	0.3930	0.2231	0.2231	0.3216	0.2231	0.4308	0.5534	0.3216	0.2877	0.3216	0.2877	0.4308	0.0000	0.0000	0.0253	****	0.9750	0.9750	0.9500
19	0.2549	0.1625	0.4308	0.1924	0.1924	0.2877	0.2549	0.4700	0.5108	0.3567	0.3216	0.3567	0.3216	0.4700	0.0253	0.0253	0.0000	0.0253	****	1.0000	0.9750
20	0.2549	0.1625	0.4308	0.1924	0.1924	0.2877	0.2549	0.4700	0.5108	0.3567	0.3216	0.3567	0.3216	0.4700	0.0253	0.0253	0.0000	0.0253	0.0000	****	1.0000
21	0.2877	0.1335	0.4700	0.2231	0.2231	0.3216	0.2231	0.4308	0.5534	0.3216	0.3567	0.3930	0.3567	0.5108	0.0513	0.0513	0.0253	0.0513	0.0253	0.0253	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

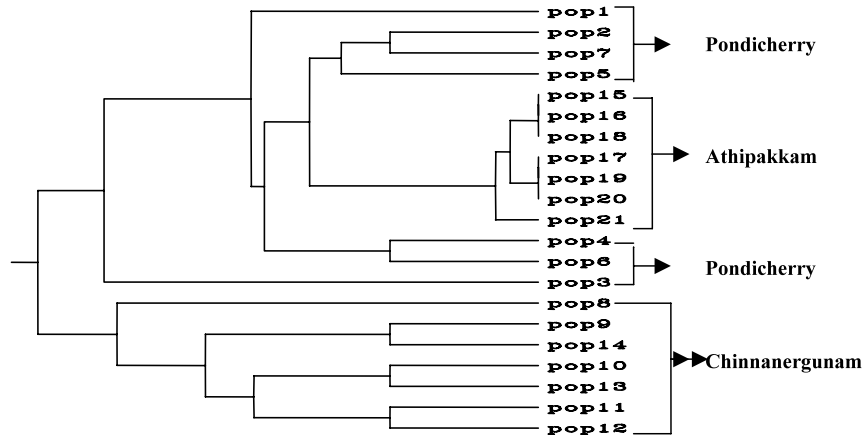


Fig. 3. Phylogenetic tree (UPGMA) of individual populations of *W. bancrofti* from South India (pop 1 to 7—Pondicherry, pop 8 to 14—Chinnanergunam & pop 15 to 21—Athipakkam).

genetic diversity. However, heterogeneity observed in the rural population, Chinnanergunam where human migration is comparatively lesser needs further investigation. Both these localities have been under DEC therapy for variable periods and it is possible that the DEC drug pressure on the parasite population would have played a role in increasing the gene diversity among the populations. When an anthelmintic drug is introduced into a parasite population selection of alleles encoding resistance to this drug increase in their frequency in the population until the drug efficacy is affected. DNA-based technologies could identify these changes. (Anderson et al., 1998). The greater gene diversity recorded in these two populations (Pondicherry and Chinnanergunam) could be attributed to this phenomenon. Another population from a distant village, where human migration is relatively restricted and active chemotherapy was unknown at the time of sampling, minimum gene diversity was observed. This region was never reported endemic for filariasis in records of National Filariasis Control Authorities and was detected endemic recently (during 1999) under a survey on WHO project on rural Bancroftian filariasis in Villupuram District of Tamil Nadu. Also, there was no chronic filariasis patients reported in the village, confirming its recency of endemicity.

Interestingly, the phylogenetic tree constructed using genetic distance of the parasite populations

exhibited a different pattern indicating the existence of two distinct clusters. While one cluster included the heterogeneous urban population and the distant homogenous rural population, the other included the heterogeneous rural population. This indicates existence of at least two genetic variants of the *W. bancrofti* populations. The rural homogeneous population of the former cluster may have originated from the heterogeneous urban parasite population. This inference was drawn from a closer observation of the genetic tree (Fig. 3) where the ancestral stock of former genetic cluster branches into two, one with only population 3 (urban Pondicherry) and the other branching out to include all the other urban populations (Pondicherry) as well as the distant homogeneous rural population (Athipakkam).

More studies on *W. bancrofti* populations from different regions are necessary to confirm the findings of the present study. Large scale studies involving more genetic markers will lead to the

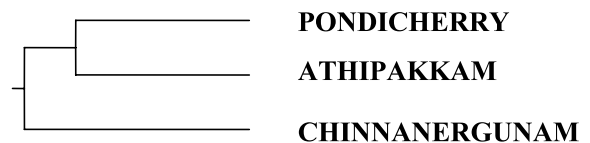


Fig. 4. Phylogenetic tree (UPGMA) of *W. bancrofti* populations grouped based on the localities of sampling (pop 1—Pondicherry, pop 2—Chinnanergunam and pop 3—Athipakkam).

identification of strains of the parasite which would be helpful in explaining the reported differences in the clinical spectrum as well as the difference in drug response of Bancroftian filariasis.

However, the initial findings of this study on the genetic variability among the *W. bancrofti* populations have important implications in the control/elimination programs of filariasis, a major tropical disease, afflicting mankind. The present study revealed the existence of genetically variant parasite populations of *W. bancrofti*. However, they are treated as a single entity chemotherapeutically as well as epidemiologically, in the control programs being undertaken on a global scale. This generates a factor of ambiguity in the success of such large-scale control programs. Also, the present study revealed the existence of greater gene diversity in the populations under chemotherapeutical operations for variable periods. Hence, the possible development of higher tolerance to the drug of choice for Bancroftian filariasis (DEC) by some genetic variants (until it is delineated) may become a causative factor for non-realization of goals of control/elimination programs.

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