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# Microsatellite analysis reveals genetic structure of *Leishmania tropica*

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**Abstract**

The current rapid spread of leishmaniases caused by *Leishmania tropica* and the complexity of its clinical spectrum call for this parasite's epidemiological and evolutionary investigation. Evaluation of its population structure by isoenzyme electrophoresis and previous molecular biological analysis has proved difficult. In this study, we used 21 microsatellite loci to type 117 strains from different African and Asian locations. Eighty-one different genotypes were found. A genetic bottleneck supported by a gradient in the number of alleles and consistent with the geographical structure of the Middle East suggests an African origin of this species. A Bayesian approach identified 10 genetic clusters that correlated predominantly with geographical origin. The strains in the 'Asia' cluster form a very heterogeneous sub-population, with a varied but inter-related genotype that is geographically very widely dispersed and consistent with anthroponotic transmission of the parasite. The other nine clusters were more homogenous. The propagation of *L. tropica* appears to be predominantly clonal. In Africa and the Middle East, anthroponotic and zoonotic systems of distribution may contribute to the development of overlapping, genetically distinct populations of *L. tropica*.


*Keywords:* Leishmania tropica; Microsatellite markers; Population structure; Life cycle; Geographical distribution

## 1. Introduction

*Leishmania tropica* is a particularly interesting and vexing parasitic species. It is very heterogeneous, displaying serological (Jaffe et al., 1990; Jacobson et al., 2003; Schnur et al., 2004), biochemical (Le Blancq and Peters, 1986; Rioux et al., 1990; Pratlong et al., 1991; Mebrahtu et al., 1992) and genetic (Schönian et al., 2001) heterogeneity. It is also associated with a broad and complex clinical spectrum of disease represented by numerous cases of simple cutaneous leishmaniasis (CL) manifesting as either single or multiple lesions, relatively rare cases of leishmaniasis recidivans (LR), rarer cases of visceral leishmaniasis (VL), including some that have also displayed post kala azar dermal leishmaniasis (PKDL) after treatment, and even an occasional case of oro-nasal and naso-pharyneal leishmaniasis (OL) (Schnur et al., 1981; Magill et al., 1994; Sacks et al., 1995; Schnur and Greenblatt, 1995). It occurs in tropical and sub-tropical regions of the Old World where its geographical distribution is very wide. *Leishmania tropica* is transmitted by at least three phlebotomine species of sand fly: *Phlebotomus (Paraphlebotomus) sergenti* in Saudi Arabia (Al-Zahrani et al., 1988), Morocco (Guilvard et al., 1991), Afghanistan (Killick-Kendrick et al., 1995) and Israel (Schnur et al., 2004); *Phlebotomus (Adlerius) arabicus* in Israel (Jacobson et al., 2003) and *Phlebotomus guggisbergi* in Kenya (Lawyer et al., 1991). Another, putative, vector for *L. tropica* in Kenya is *Phlebotomus aculeatus* (Johnson et al., 1993).

The epidemiology of leishmaniases caused by *L. tropica* has not been elucidated fully. Disease is often described as being urban and anthroponotic and most infections occur in and around quite densely populated cities. However, the paucity of cases in some foci and sudden occurrence of small outbreaks of

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disease in semi-rural locations suggest that disease may be zoonotic in some cases (Mebrahtu et al., 1992; Klaus et al., 1994; Sang et al.; Kamhawi et al., 1995; Guessous-Idrissi et al., 1997; Jacobson et al., 2003; Schnur et al., 2004). Strains of *L. tropica* have been isolated from dogs in Morocco (Dereure et al., 1991) and in Kenya hyraxes are a putative animal reservoir host (Sang et al., 1994). Further, a study using a PCR of the internal transcribed spacer region 1 carried out on tissues from three hyraxes caught in Israel just north of the Sea of Galilee yielded DNA products characteristic for *L. tropica* (Jacobson et al., 2003) and a strain of *L. tropica* was cultured from a fourth hyrax (Jaffe et al., 2004).

The high degree of heterogeneity of *L. tropica* was suggested to be associated with genetic exchange (Pratlong et al., 1991). It is of medical and economic importance to know if its propagation is strictly clonal or panmictic. Recently, there has been an upsurge of cases of CL caused by *L. tropica*, including the emergence of several new foci in Morocco (Rhajaoui et al., 2004), Kenya (Johnson et al., 1999), Ethiopia (Gebre-Michael et al., 2004), Israel (Jacobson et al., 2003; Schnur et al., 2004), The Palestinian Authority (Al-Jawabreh et al., 2004), Jordan (Kamhawi et al., 1995), Iran (Yaghoobi-Ershadi et al., 2002) and Afghanistan (Reithinger et al., 2003). The rapid spreading of the parasite with increasing rates of infection begs epidemiological investigation. It is of interest to clarify the situation of strains from Namibia and some of those from Tunisia with characteristics similar to *L. tropica*, which have been proffered as new species (Lanotte G., Rioux J. A., Serres E., 1986. Approche cladistique du genre *Leishmania* Ross, 1903. A propos de 192 souches originaires de l'Ancien Monde. Analyse numerique de 50 zymodemes identifies par 15 enzymes et 56 isoenzymes. Pp. 269–288 in: *Leishmania*. Taxonomie et Phylogenese. Applications eco-epidemiologiques. (Colloque International CNRS/INSERM/OMS 2-6 Juillet 1984), Montpellier: IMEEE; Rioux, J. A., Lanotte G., Pratlong F., 1986. *Leishmania killicki* n.sp. (*Kinetoplastidae, Trypanosomatidae*). Pp. 139–142 in: *Leishmania*. Taxonomie et Phylogenese. Applications eco-epidemiologiques. (Colloque International CNRS/INSERM/OMS 2–6 Juillet 1984), Montpellier: IMEEE; Van Eys et al., 1989; Rioux et al., 1990).

None of the methods revealing the heterogenic character of *L. tropica* have shown a clear idea of the population composition for this species. Revealing the genetic composition and population structure through genetic analysis of this species based on co-dominant genetic markers like microsatellites should help to give clues to its heterogeneity and propagation. Microsatellite markers have been used in numerous population studies (Russell et al., 1999; Chambers and MacAvoy, 2000; Sunnucks, 2000; Toth et al., 2000; Wirth and Bernatchez, 2001; Bulle et al., 2002) and have been shown to be a useful tool in determining genetic distances between individuals and groups of individuals. Development of microsatellite markers suitable for the epidemiological and genetic investigations of *L. tropica* was recently described (Schwenkenbecher et al., 2004), and a further number of markers is available from a set that was developed for *Leishmania donovani* (Jamjoom et al., 2002, 2004). Here, 117 strains of *L. tropica* from different geographical regions were genotyped using microsatellite markers. The data set was analysed with the aim of unravelling the population structure of the parasite and the genetic relationships of the strains.

## 2. Material and methods

### 2.1. Source of parasites

Thirty-three strains of *L. tropica* used in this study were from Turkey, 27 from The Palestinian Authority, 21 from Israel, nine from Morocco, seven from Kenya, five from Azerbaijan, four from Namibia, four from India, three from Iraq, two from Tunisia and one each from Jordan and Egypt (Table S1). The strains were obtained from: the Department of Biomedical Research, the Royal Tropical Institute, Amsterdam, The Netherlands; the WHO's Reference Centre for the Leishmaniases, the Hebrew University, Jerusalem, Israel; the Centre National de Référence des *Leishmania*, Université de Montpellier I, France; the Department of Molecular Biology, the Lomonossov State University, Moscow, Russia; The Islah Charitable Social Society, Jericho, The Palestinian Authority and the Department of Biochemistry, Al-Quds University, Abu Dis, The Palestinian Authority. The strain *Leishmania major* MHOM/IL/1980/Friedlin used as an out-group was also obtained from the WHO's Reference Centre for the Leishmaniases, Jerusalem.

### 2.2. DNA extraction and microsatellite genotyping

DNA was extracted as described by Schönian et al. (1996). Microsatellite analysis was performed using 21 microsatellite markers developed and optimized for *L. tropica* (Schwenkenbecher et al., 2004) and *L. donovani* (Jamjoom et al., 2002). Microsatellite amplification and fragment size analysis by either polyacrylamide electrophoresis or fluorescence detection in an automated sequencing system (Beckman Coulter) was done as described by Schwenkenbecher et al. (2004).

### 2.3. Genetic distance and genotyping

The entire dataset was clone-corrected for all analyses except for the generation of the Neighbour-Joining tree.

Population structure was investigated using the Bayesian model-based clustering approach implemented in STRUCTURE (Pritchard et al., 2000). On the basis of multilocus data, this approach places individuals into K populations. Individuals can be assigned to multiple clusters with the membership coefficients of all the clusters summing up to one. A burnin period of 20,000 iterations was set and probability estimates were obtained, using 200,000 Markov Chain Monte Carlo iterations. The most appropriate number of populations was determined by comparing log-likelihoods (ln Pr($K$/$X$), where $X$ is the number of genotypes) for values of $K$ between 1 and 15. On comparing the values graphically, the resulting curve had a Gaussian distribution. At the maximum, the plateau, the value of $K$ encompassed the main structure embodied in the data.