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MINI-REVIEW

Molecular markers for *Phlebotomus papatasi* (Diptera: Psychodidae) and their usefulness for population genetic analysis

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Summary Three molecular typing tools: multilocus microsatellite typing, *cytochrome b* sequence analysis and internal transcribed spacer 2 (ITS2) sequence analysis, were evaluated for their usefulness in inferring the population structure of *Phlebotomus papatasi* sand flies. ITS2 sequence analysis did not prove suitable for inferring phylogenetic and population genetic relationships across *P. papatasi* sand flies. Microsatellite markers showed high resolution in differentiating globally distributed *P. papatasi* populations, whereas *cytochrome b* sequence analysis provided insight into the relationships between closely related populations from the Mediterranean. Population structure, differentiation and demographic history among *P. papatasi* are important for understanding patterns of dispersal in this species and for planning appropriate control measures.

Phlebotomus papatasi is the main vector of *Leishmania major*, which is one of the causative agents of Old World zoonotic cutaneous leishmaniasis. It has a large geographical distribution, ranging from Morocco to the Indian subcontinent and from southern Europe to central and eastern Africa. The geographical range of *P. papatasi* includes varying climatic and ecological discontinuities. Therefore, knowledge of the genetic relationships and biogeography among *P. papatasi* populations would enable better understanding of the current geographic distribution and improve the design of appropriate control measures.

Population genetic studies should rely on markers that have appropriate discriminatory power, depending on the question being asked. Markers that are neutral, not under selective pressure and are co-dominant are highly significant in population studies. Multilocus enzyme electrophoresis and several approaches based on DNA sequence comparisons fulfil these requirements, for example, the sequencing

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of mitochondrial genes such as *cytochrome b* (*cyt b*) and of different housekeeping genes by multilocus sequence typing, the typing of single nucleotide polymorphisms and the exploration of variation in microsatellite sequences with multilocus microsatellite typing (MLMT).

Genetic studies on *P. papatasi* sand flies are still very scarce. Various *cyt b* haplotypes have been identified in *P. papatasi* populations from different Mediterranean areas.[1] Depaquit studied the relationships between different sand fly species by analysing rDNA sequences.[2] This mini-review highlights recent findings obtained by analyzing large numbers of *P. papatasi* individuals originating from widely separated populations, allowing the re-assessment and re-evaluation of well-known genetic typing techniques as well as the newly developed multilocus microsatellite typing tool.

The sequence typing of rDNA spacers has many advantages, such as enormous variability due to high mutation rates and high sensitivity due to their multi-copy character. Analysis of internal transcribed spacer 2 (ITS2) sequences revealed significant intra-specific variation for the *P. papatasi* individuals studied and even intra-individual variation in the multiple copies of the ribosomal spacer. It seemed that variant copies of ITS2 within individual sand flies often differed as much as between flies from different localities. This resulted in poor geographical structuring. Thus, ITS2 sequences were found to be too variable to resolve the genetic relationships between different populations of *P. papatasi*. Therefore, ITS2 does not represent a suitable marker for inferring phylogeny and population genetics in *P. papatasi* sand flies.[3]

MLMT[4] and comparisons of mitochondrial *cyt b* sequences[1,5] have proven useful for the detection of intra-species variation in *P. papatasi*. Consistently significant levels of genetic differentiation have been observed using both tools. MLMT revealed the existence of population structuring and sub-structuring and allowed globally isolated populations to be distinguished. The comparison of *cyt b* gene sequences provided important information concerning *P. papatasi* populations and their demographic history. The technique was useful in resolving the genetic relationships of closely related populations of *P. papatasi*; five populations were distinguished in the Mediterranean area and the Middle East, and two populations were found to co-exist in Palestinian and Israeli foci.[1] There are many reasons why *cyt b* is often used for phylogenetic analysis in sand flies. The high discriminatory power of the *cyt b* marker is based on the existence of discrete character classes (i.e. the three codon positions) that exhibit mutation rates reliable for phylogenetic analysis, plus the fact that the gene is maternally inherited and, thus, free of recombination. Furthermore, the gene evolves slowly in terms of non-synonymous substitutions, but the rate of silent mutations is relatively high. Sequence variation in *cyt b* was due only to single point mutations in all *P. papatasi* individuals studied.

The slow mutation rate and absence of recombination of the *cyt b* marker seems to make it better suited than microsatellites for differentiating closely related populations and distinguishing populations in close geographical proximity. Using MLMT in conjunction with comparison of *cyt b* sequences is highly recommended to provide dual insights into the population structure of *P. papatasi*.

Using more microsatellite markers could be promising for better resolution of closely related populations. Sequencing the *P. papatasi* genome is of great significance to map the whole genome for the existence of potential markers on both population and specific levels.

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Ethical approval: Not required.

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