

# Sand Fly Evolution and its Relationship to *Leishmania* Transmission

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*The evolutionary relationships of sand flies and Leishmania are discussed in this report, which draws distinctions between co-association, co-evolution and co-speciation (or co-cladogenesis). Examples focus on Phlebotomus vectors of Le. infantum and Le. major in the Mediterranean subregion.*

Key words: co-evolution - *Phlebotomus* - *Leishmania*

There is a noticeable association between certain Old World species or species complexes of *Leishmania* and particular subgenera of *Phlebotomus*, as characterized by isoenzymes and morphology respectively. The term "evolutionary fit" has been used (Killick-Kendrick 1985), but Essegir et al. (2000) concluded that clear distinctions have not always been drawn between co-association (with the transmission cycle having a distinctive "landscape epidemiology"), co-evolution (e.g. between molecules of the sand fly midgut and the parasite external surface) and vector-parasite co-speciation (or co-cladogenesis).

The evolutionary relationships between sand flies and *Leishmania* would seem to have implications for leishmaniasis control or intervention. For example, putative vectors in previously unexplored foci can usually be targeted simply by their taxonomic relationship to known vectors. In the Mediterranean subregion, western Asia and sub-Saharan Africa all the firmly incriminated vectors of *Le. major* (but not some of its suspected vectors) are classified in the subgenus *Phlebotomus* and, similarly, most of the firmly incriminated vectors of *Le. tropica* and *Le. infantum* are placed in the subgenera *Paraphlebotomus* and *Larroussius*, respectively (Killick-Kendrick 1990). These might be longstanding evolutionary relationships, in which taxa of parasites and vectors are locked together by unique behavioural or molecular phenotypes of epidemiological importance. Thus, only *P. (Phlebotomus) papatasi* would seem to have the midgut molecules to which the highly substituted side-chains of the lipophosphoglycans (LPGs) of *Le. major* can bind (Pimenta et al. 1994). However, too few parasite-vec-

tor combinations have been experimentally investigated for one to conclude that ligand-receptor co-evolution in the midgut is the key to the association between *Le. major* and the subgenus *Phlebotomus*. The determinants of the arid-habitat associations of the vectors and the gerbil reservoir hosts might also be critical. So far, based on cytochrome b sequences of sand fly mitochondrial DNA (mtDNA), there is no evidence for co-cladogenesis between the four species in the subgenus *Phlebotomus* and zymodemes of *Le. major* (Essegir et al. 1997). The same zymodemes have been isolated from *P. duboscqi* south of the Sahara and from *P. papatasi* in the Mediterranean subregion. The geographical populations of *P. papatasi* are separated by small genetic distances in a way consistent with a recent radiation from the eastern Mediterranean Basin to north Africa, southern Europe and western Asia (Essegir et al. 1997), but there is no clear evidence for a similar radiation of *Le. major* strains.

There has been speculation about the evolutionary relationships between *Phlebotomus* of the subgenus *Larroussius* and *Le. infantum*, perhaps because some strains of this parasite have been associated with cutaneous rather than visceral human disease. The mode of speciation of Mediterranean *Larroussius* has recently been inferred from comparative sequence analyses not only of mtDNA but also of a nuclear gene, elongation factor alpha (Essegir et al. 2000). The molecular phylogenies were congruent basally, where their clades matched species complexes defined by a few genitalic characters of each sex. For the most derived species complex (*P. perniciosus*), however, there was strong evidence for reticulate evolution. The molecular phylogenies were incongruent and mtDNA marker distribution was consistent with introgressive hybridizations between species whose current ranges overlap or abut. Reticulate evolution in the *P. perniciosus* complex (Pesson et al. 1999, Ready & Pesson 1999) makes it difficult to detect co-cladogenesis with *Leishmania*.

Essegghir et al. (2000) considered not only the molecular phylogenies but also the ecological niches of the *Larroussius* species and the historical biogeography and palaeoecology of the Mediterranean subregion. They concluded that there had been sequential speciation (a "taxon pulse") promoted by increasing aridification in the Pliocene, later than previously proposed but too early for Pleistocene Ice-age refugia to have played a part. Speciation would have occurred before the proposed divergence of *Le. donovani* and its sister species *Le. infantum*, and this helped to rule out any strict vector-parasite co-speciation or co-cladogenesis with strains.

The conclusions of Essegghir et al. (2000) depended on a molecular clock for mtDNA. The sand fly fossil record is poor (Lewis 1982), and so proposals concerning the speciation of sand flies should be supported by molecular-clock datings allied to resolved (molecular) phylogenies, rather than general hypotheses about vicariance and tectonic activities. The dating of the divergence of *Le. donovani* and *Le. infantum* was based on an isoenzyme clock (Moreno et al. 1986), and this should be compared with DNA clocks.

The absence of any unequivocal evidence for strict co-cladogenesis in the species-level examples considered above emphasizes the need for more precise biological explanations of the strong associations between some groups of sand flies and *Leishmania*. Inferences based on phylogenetic relationships can be used to formulate interesting evolutionary hypotheses, but these should be tested experimentally. For example, is the structure of the LPG of *Le. major* a critical factor for explaining the ability of all members of the subgenus *Phlebotomus* to transmit this parasite, or is *P. papatasi* (the only species investigated experimentally) unique in this respect?

The evolution of the molecules involved in vector-parasite specificity may not always be directly linked to the speciation (or cladogenesis) of their hosts, especially if there is gene introgression. In this respect, it is becoming clear that interspecific hybridization and gene introgression can be expected among recently evolved sand fly morphospecies, in the American genus *Lutzomyia* (Marcondes et al. 1997) as well as in the *Phlebotomus* subgenera *Larroussius* and *Phlebotomus*, for which there is experimental evidence of interbreeding (see Essegghir et al. 1997).

Co-cladogenesis, and its epidemiological significance, should be more easily discernible among higher taxonomic groups, after the completion of "lineage sorting" aided by reproductive barriers. Molecular data are helping to define some of these higher taxonomic groups. For example, based on

small subunit nuclear ribosomal RNA gene sequences, it is clear that most (if not all) *Phlebotomus* vectors of parasites in the *Le. donovani* complex belong to one monophyletic group, which includes sand flies in the subgenera *Euphlebotomus* and *Adlerius* in addition to *Larroussius* (Aransay et al. 2000).

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