

ESTABLISHING INSECTICIDE RESISTANCE ANALYSIS IN SAND FLIES

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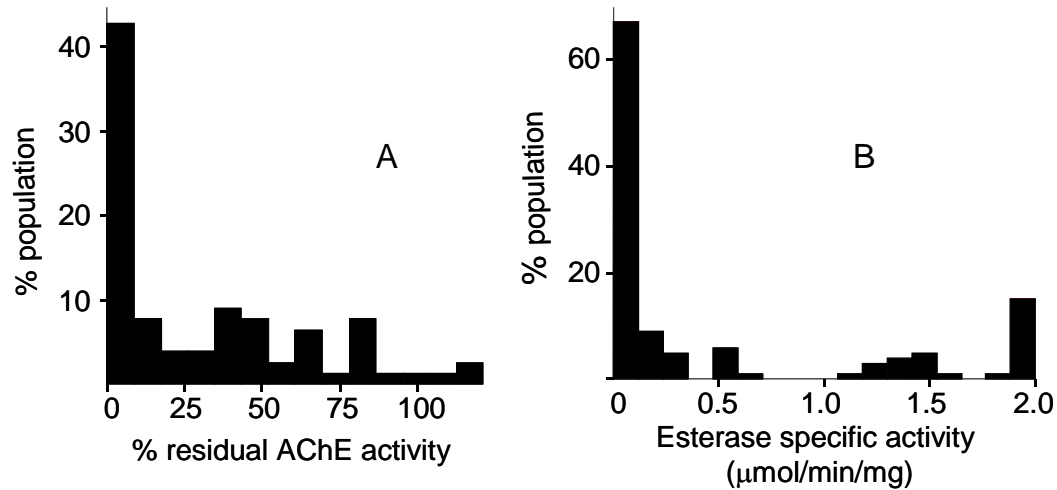
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Leishmaniasis currently affects approximately 12 million people in 88 countries with 350 million at risk worldwide, and is increasing, in part as a result of increased movement of disease naïve populations due to unrest in desert and semi-desert areas. The parasite is transmitted by sand flies: predominantly *Phlebotomus* species in the Old World and *Lutzomyia* species in the Americas. Globally, sand fly populations are still largely susceptible to chemical control, and historically there have been reports of the interruption of leishmaniasis transmission due to anti-mosquito control interventions. Resistance has been detected in sand flies by WHO bioassays, but there are no biochemical or molecular studies of the underlying insecticide resistance mechanisms to date. Such information is essential in designing appropriate control measures, since different mechanisms confer cross-resistance between insecticide classes.

With an increasing incidence of cutaneous leishmaniasis in Sri Lanka, particularly in northern provinces, insecticide-mediated vector control is under consideration. Optimising such a strategy requires the characterisation of sand fly populations in target areas with regard to species composition and extant resistance, among other parameters. We have recently completed a small study of a sand fly population from Delft Island off the northwest coast of Sri Lanka. Delft Island is used as an illegal transit location by many refugees returning to the north of Sri Lanka from southern India, where leishmaniasis is endemic.

Sand flies were collected by human bait and cattle baited net traps. One aim of the study was to investigate the potential for using DNA-based methods of species identification. Genomic DNA was extracted and a fragment of the ribosomal 18S gene amplified. The sequence from all flies analysed matched that of *Phlebotomus argentipes* Annandale & Brunetti, the primary leishmaniasis vector in India and the most likely vector in Sri Lanka. Independent morphological analysis also identified *P. argentipes*.

To establish the current insecticide susceptibility status of sand flies from Delft Island, we analysed the collection for the activities of 4 enzyme systems involved in insecticide resistance (acetylcholinesterase, non-specific carboxylesterases, glutathione-S-transferases and cytochrome p450 monooxygenases), adapting procedures established for use with mosquitoes. There was preliminary evidence for elevated esterases and altered acetylcholinesterase in this population, which probably arose from the malathion-based spraying regimes of the Anti-Malarial Campaign.



Distribution of enzyme activities in Delft Island sand flies.

- A. Acetylcholinesterase activity after inhibition with propoxur as a proportion of activity without inhibition.
- B. Esterase activity.

This is the **first study** to identify these mechanisms in any sand fly. The increasing likelihood of chemical control targeted specifically against sand flies, whether through impregnated dog-collars or residual spraying, necessitates further, molecular, investigation of these mechanisms. Our preliminary data establish baselines against which subsequent collections can be evaluated, and suggest that sand fly control by OPs and carbamates in this area may have already been compromised.

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