SUMMARY - RG/93/BT/2

Title: Characterization of *Anopheles culicifacies* complex of Sri Lanka; Identification of sibling species using DNA probes.

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Period of contract : November 1993 - December 1997

Background and Scope of project:

Anopheles culicifacies is the best known vector of malaria in Sri Lanka. The taxon *Anopheles culicifacies* exists as an isomorphic species complex comprsing of four reproductively isolated populations designated as A, B, C and D. The four species have been found to be different in their pattern of distribution, behaviour, and in vector competency, and therefore may play a different role in malaria transmission. Precise identification of members within a taxon is thus essential for the successful implementation of vector control programmes. The current cytotaxonomic methods of identification of sibling species of *An. culicifacies* complex are tedious and are sex and life cycle stage specific. Therefore, a large number of samples cannot be identified due to unsuitable preparations. The use of DNA probes is an alternative novel approach for vector identification due to the accuracy, sensitivity, reliability and simplicity offered by the technique. This study describes the development of DNA probes for the *An. culicifacies* species complex.

Experimental Method:

Cloning of repetitive DNA sequences of the *An.culicifacies* genome was carried out by a colony hybridization assay with a genomic library of *An.culicifacies* species B constructed in pUC19 vector and radiolabelled total *An.culicifacies* (B) DNA as a probe. The recombinant plasmids of clones which gave a strong hybridization signal were isolated for further analysis. The purified cloned DNA inserts of these clones were tested for their specificity and sensitivity as ^{32}P radiolabelled and biotinylated probes for the identification of the *An.culicifacies* species complex using dot blot and squash blot hybridization assays. The cloned sequences were characterized by DNA sequencing.

Results obtained :

Three highly repetitive sequences Rp36, Rp217 and Rp234, were isolated from the *An. culicifacies* genome. The cloned DNA sequences were specific to *An.culicifacies* species and were found at a higher copy number in sibling species B and C than in species A and may therefore be used as radiolabelled DNA probes to distinguish species A from B and C species, using a 200-fold dilution of a single mosquito DNA extract in a dot blot hybridization assay. The probes also could be used to detect adult and immature stages *of An.culicifacies* from those of other mosquito species. A simple and safe mosquito head-squash blot- hybridization assay was further developed to detect sibling species A from B and C species using biotinylated Rp217 probe. Screening of 2155 wild caught *An. culicifacies* mosquitoes with these probes revealed the absence of species A in Sri Lanka. Rp36 and Rp217 have been completely sequenced. Internal repeats were absent in Rp36 and two related core sequences of 13 and 16 bp were found tandemly repeated in Rp217.

Conclusions

The probes developed in this study enable the rapid detection of all life cycle stages of *An. culcifacies* from other mosquito species, as well as the detection of *An.culicifacies* sibling species A from B and C sibling species in the field. Field investigations using these DNA probes revealed the absence of *An.culicifacies* sibling species A in Sri Lanka. The probes Rp36 and Rp217 have been completely sequenced.

Publications and Communications

Gunasekera, M.B., De Silva, B.G.D.N.K., Abeyewickreme, W. and Karunanayake, E.H. (1993) Characterization of DNA probes that detect sibling species A *of Anopheles culicifacies* complex. Proc. Sri Lanka Asso. Adv. Sci. 49: 135.

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De Silva, B.G.D.N.K., Ratnayake, W.E., Gunasekera, M.B., Abeyewickreme, W and Karunanayake, E.H. and Wickramasinghe, M.B. (1993). Colonization and characterization of *Anopheles culicifacies* Giles, the major malaria vector in Sri Lanka. Mosquito-Borne Diseases Bulletin 10: 115.

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de Silva, B.G.N.K., Gunasekera, M.B., Abeyewickreme, W., Nanadadasa,H.G. and Karunanayake, E.H.(1996) A simplified non-radioactive DNA probe for the field detection of sibling species A of the *Anopheles culicifacies* complex. Proceedings of the Global Meet on Parasitic Diseases, New Delhi, India, 81p.

The following Manuscripts have been submitted for publication.

de Silva, B.G.N.K., Gunasekera, M.B., Abeyewickreme, W., Townson, H., and Karunanayake, E.H.(1998) A simplified non-radioactive DNA probe technique for the field detection of sibling species A of the *Anopheles culicifacies* (Diptera; Culicidae) complex. (Submitted to Bulletin of Entomological Research)

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de Silva, B.G.N.K., Gunasekera, M.B., Abeyewickreme, W., Wickramasinghe, M..B. and Karunanayake, E.H.(1998) Seasonal trends of *Anopheles culicifacies* population at Gomadiyagala - a village in the North Western Province of Sri Lanka. (Submitted to Vidyodaya Journal of Science)

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