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Filariasis in Sri Lanka

II. Crossing Relations of Natural Populations of *Culex quinquefasciatus* (Say) in Sri Lanka

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Abstract : Tests on hybrid compatibilities of *Culex quinquefasciatus* (Say) in Sri Lanka were carried out using strains from nine (9) different locations. Thirty-four test crosses were performed and a total of 628 egg rafts were examined for hatchability. Percentage hatch for all test crosses were above 70.0% indicating full compatibility and homogenicity of *Cx. quinquefasciatus* populations of Sri Lanka.

1. Introduction

The common house mosquito, *Culex quinquefasciatus* (Say) is the vector of human filariasis due to *Wuchereria bancrofti* in Sri Lanka. This disease has been known to be endemic in the island in an area of 55 sq kilometers in extent, forming a narrow belt from north of Negombo to east of Matara (Figure 1). However, *Cx. quinquefasciatus* is present in all urban areas of the island. So far no studies have been made to try to elucidate the reasons why filariasis is absent in areas outside the endemic belt. The present study is to establish whether reproductively isolated strains of *Cx. quinquefasciatus* populations exist in different areas of Sri Lanka including two representative areas from the endemic belt. Comparative studies on transmission of *W. bancrofti* have been made on laboratory bred populations of *Cx. quinquefasciatus* collected from within and outside the endemic belt. (Samarawickrema *et al* - unpublished data.)

The naturally occurring phenomenon of cytoplasmic incompatibility has been well established in the *Culex pipiens* complex. Crossing types of *Culex pipiens/quinquefasciatus* from different geographical regions have been studied by Laven.[4] Laven[5] has also demonstrated the phenomenon of cytoplasmic incompatibility between different strains of *Cx. quinquefasciatus* in African populations. Genetic studies of *Cx. quinquefasciatus* carried out in WHO/ICMR unit in New Delhi have revealed, that Delhi population of *Cx. quinquefasciatus* is polymorphic for its crossing properties. This polymorphism seems to affect the compatibility of wild males with Paris and Prague females, while there is no detectable variation in the crossing properties of the wild females.[8] The majority type in this Delhi population of *Cx. quinquefasciatus* was bidirectionally incompatible with Paris strain, while minority type has males compatible, but females incompatible with Paris males.[7] The two types are however fully compatible with each other. Krishnamurthy and Laven[3]

reported that populations from several types in India were fully compatible with each other and they also found that in Bangkok, there were two cytoplasmic types within the wild *Cx. quinquefasciatus* population, one type of male being compatible with Paris females and the other being incompatible.

In the light of the above knowledge, the present study was carried out as a preliminary step to ascertain the crossing relations of *Cx. quinquefasciatus* populations within the filarial endemic belt with those existing outside the belt.

2. Materials and Methods

In this study the term strain is used for *Cx. quinquefasciatus* populations collected from each area under investigation. The areas from which collections were made are indicated in Figure 1. Peliyagoda and Matara were selected as suitable areas within the endemic belt as they have been known to have high positive rate of microfilariae in the human population over several years.[1] The areas outside the endemic belt were selected at random so as to cover the entire island (Figure 1). Kandy, Badulla, Nuwara Eliya and Ratnapura represent the hill country whereas Jaffna and Mannar represent the northern dry zone, and Batticaloa, the eastern province. Adult, blood-engorged *Cx. quinquefasciatus* females were collected from among the house resting populations in each of these areas and transported in cages to the laboratory in Colombo, where they were maintained in 30 cm cube cages draped with wet lint and allowed to become gravid. Each strain was maintained separately to obtain the the corresponding F.1 generation.

Batches of larvae of each strain were reared on matured guinea-pig dung infusion (72 hrs old) and fed with dried prawn powder. They were accurately sexed at the pupal stage and virgin adult females were used in all experimental crosses. The newly emerged adults were maintained on 10% dextrose solution inside 30 cm cube cages, draped with damp lint and covered with polythene covers to ensure high humidity.

Experimental crosses were made with F. 1 adults. All strains selected from outside the endemic belt were crossed to Peliyagoda and Matara strains. In all these studies reciprocal crosses were also made. The number of males to females in each cross was set up in ratio of 2:1 to ensure the highest percentage of insemination of virgin females.[6] In all crossing experiments, the males used were less than five days old and the virgin females 2-3 days old. Adult females were then fed on chick blood and the engorged ones maintained in a humid atmosphere until gravid. They were not fed on sugar during this period as it might have an effect on oviposition.[2] Gravid females were tubed individually for oviposition in glass tubes measuring 2.5 × 5 cm and containing dilute guinea-pig dung infusion and strips of straw in order to prevent females from drowning. The egg rafts laid were left in the tubes for hatching and individually scored for hatchability.

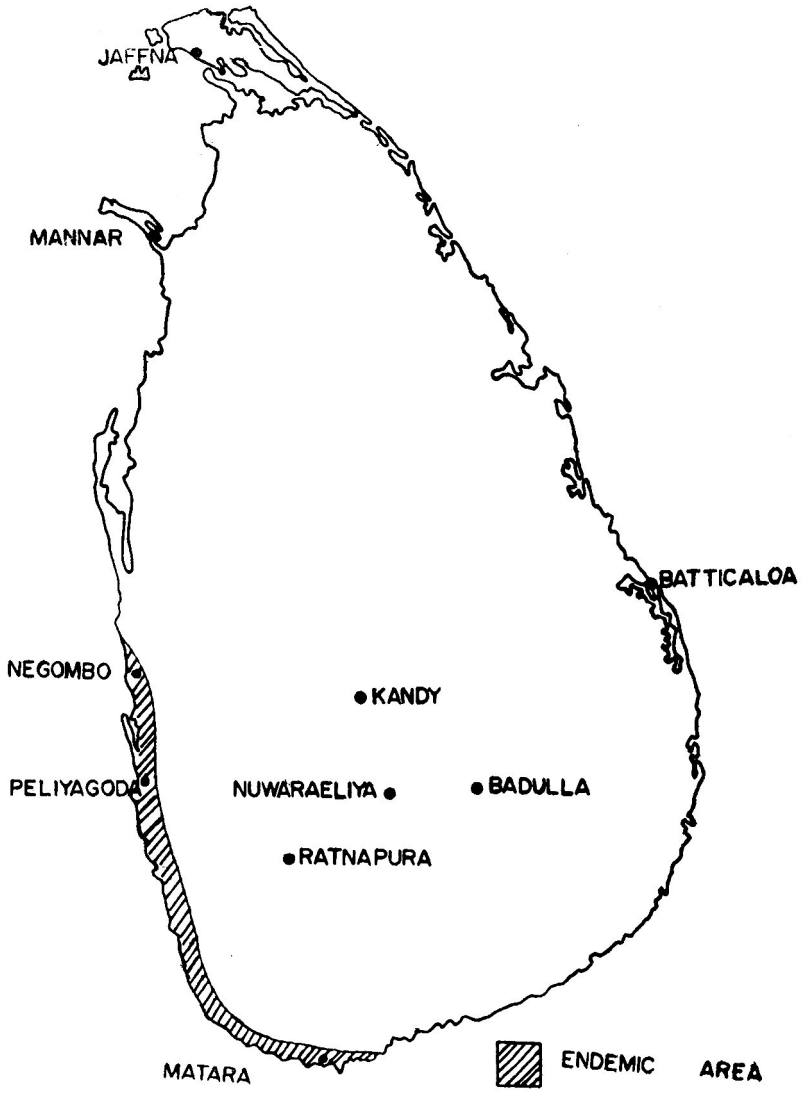

Figure 1. Map of Sri Lanka showing the urban areas from where populations of *Culex quinquefasciatus* were collected for crossing experiments.

48 hours after egg laying each raft was transferred on to a strip of filter paper placed on a clean glass slide. Then a drop of xylene was introduced on to the egg raft on the filter paper to dissolve the cement-like substance which held the eggs together. The eggs slipped out of the gelatinous matrix of the raft and remained well separated from one another. This technique afforded easy and accurate scoring of several thousands of eggs per day. The unhatched eggs were examined carefully to distinguish between the embryonated and unembryonated eggs.

3. Results and Discussion

Altogether 34 experimental crosses were carried out (Figure 2) and a total of 628 egg rafts were tested for hatchability. Except for a few experimental crosses the number of rafts examined for each cross was well over 10. In the Nuwara-Eliya females to Peliyagoda males experimental cross, one raft out of 25 rafts examined showed no hatch. Similarly, of Ratnapura females to Matara males experimental cross, one out of 24 rafts examined showed no hatch. Out of the 117 rafts examined for Peliyagoda females to Peliyagoda males, only one raft showed no hatch. These 3 rafts when examined under the stereoscopic microscope showed that all eggs were unembryonated. The respective females on dissection showed that spermathecae were negative for sperms. Percentage hatch for all crosses carried out was well above 70% indicating high compatibility between all strains of *Cx. quinquefasciatus* in Sri Lanka.

Thus, as far as crossing relations between *Cx. quinquefasciatus* populations in different areas of Sri Lanka are concerned, there is reciprocal compatibility in all populations tested. It is now intended to carry out compatibility studies between *Cx. quinquefasciatus* from Sri Lanka with other members of the *Culex pipiens* complex existing in different parts of the world to find out whether incompatibilities exist and whether Sri Lanka population of *Cx. quinquefasciatus* exhibits polymorphism similar to that in Indian populations of *Cx. quinquefasciatus*.

During the course of the present study, although all strains tested were reciprocally compatible, indicating no apparent post-copulatory isolating mechanisms between *Cx. quinquefasciatus* populations in Sri Lanka, we noticed certain remarkable biological differences between the adult populations of *Cx. quinquefasciatus* collected from different areas. Firstly, the general size and the appearance of adults collected from each area; the adults collected from Nuwara Eliya were quite large and robust in appearance when compared with Mannar or Jaffna strains. One striking factor in these two areas is the extreme climatic conditions; that is, Nuwara Eliya being situated in the central hill country of Sri Lanka, where the climate is cool and humid throughout the year, as compared to the warm and arid climate prevailing in Jaffna and Mannar which are situated along the coast in northern zone of Sri Lanka.

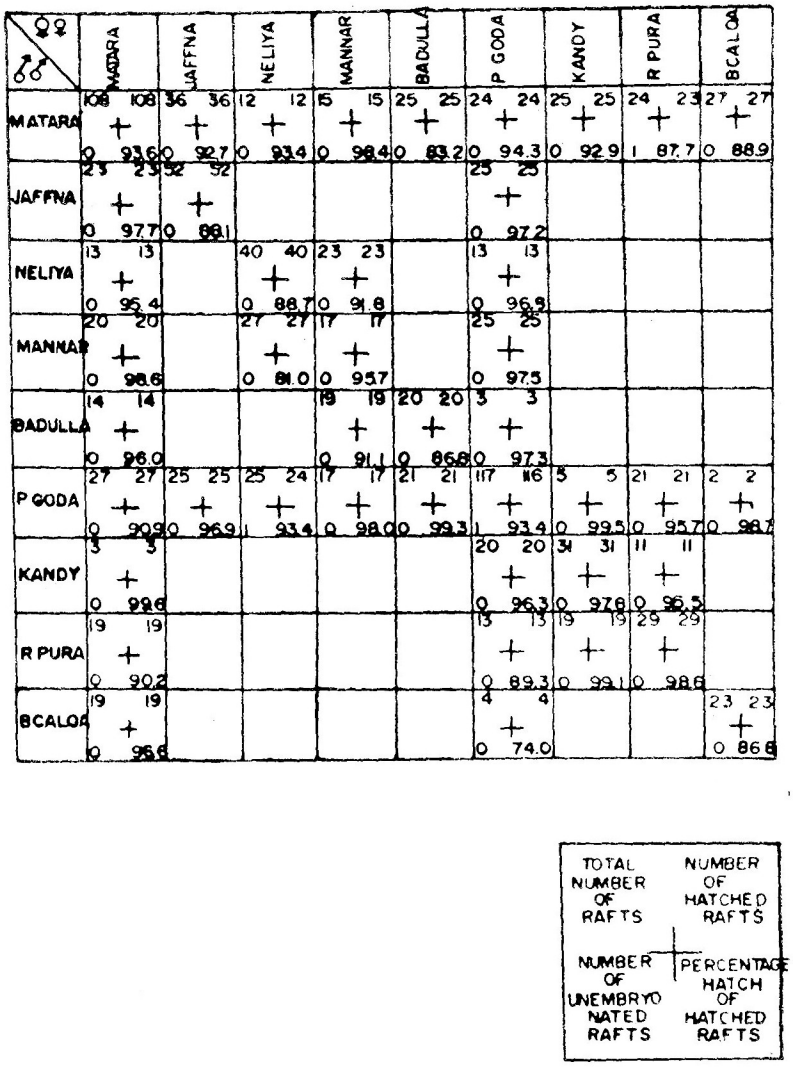

Figure 2. Results of crosses between different populations of *Culex quinquefasciatus* in Sri Lanka.

Studies carried out on fecundity of these different populations also showed a variation for each population. A higher fecundity for F. 1 females from Nuwara Eliya was observed when compared with those of Mannar and Jaffna.

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References

1. ABDULCADER, M. H. M. & SASA, M. (1966). *Epidemiology of Filariasis in Ceylon. Jap. J. exp. Med.*, **36:** 609-630.

2. DE MEILLON, B., SEBASTIAN, A. & KHAN, Z. H. (1967). *Cane-sugar feeding in Culex pipiens fatigans. Bull. W.H.O.* **36:** 53-65

3. KRISHNAMURTHY, B. S. & LAVEN, H. (1976). *Development of cytoplasmically incompatible and integrated (Translocated incompatible) strains of Culex pipiens/fatigans for use in genetic control. J. Genet.* **63:** 117-129.

4. LAVEN, H. (1967). *Incompatibility in Culex pipiens. Genetics of Insect Vectors of Disease* 256-268, eds. J.W.Wright and R. Pal. Elsevier, Amsterdam.

5. LAVEN, H. (1969). *Incompatibility tests in the Culex pipiens complex*, Part 1, African strains. *Mosquito News*, **29:** 70-74.

6. SEBASTIAN, A. & DE MEILLON, B. (1967). *Experiments on the mating of Culex p. fatigans in the Laboratory. Bull. W.H.O.* **36:** 47-52.

7. SUBBARAO, S. K., CURTIS, C. F., SINGH, K. R. P. & KRISHNAMURTHY B. S. (1974). *Variation in cytoplasmic crossing type in populations of Culex p. fatigans from the Delhi area. Journal of Communicable Diseases.* **6:** 80-82.

8. SUBBARAO, S. K., KRISHNAMURTHY, B. S., CURTIS, C. F., SINGH, K. R. P., ADAK, T. & CHANDRAHAS, R.K. (1977). *Further studies on variation of cytoplasmic incompatibility in the Culex pipiens complex. Indian J. Med. Res.*, **65 :** (Suppl.) 21-33.