LABORATORY COLONISATION OF MANSONIA MOSQUITOES

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MOSQUITOES stems from the fact that they are vectors of Brugian filariasis in Kerala State. Laboratory rearing of Mansonia has been reported by Gillett, Jayawickreme and Niles and Laurence et al. However, owing to the nonavailability of "paper leaves" used by Laurence et al it became necessary to search for suitable materials for larval attachment and newer techniques had to be devised for laboratory rearing of Ma. annulifera and Ma. uniformis.

In the present study two different methods were used for the rearing of Mansonia mosquitoes in the laboratory. In the first, adults collected from the field were released in small cages of 18" x 18" x 22". A small bowl containing water and P. striatipes or thermocoll (an expanded polystyrene, commonly used as packing material) were kept in the cage for oviposition. Eggs were removed from the bowl and kept in wide-mouthed plastic containers (2000 ml capacity) with dechlorinated tap water. In order to prevent overcrowding only 2 egg clusters were kept in one container. P. plants were provided for larval attachment and these were replaced by fresh ones as and when they decayed. The emerged adults were released back in the cage. In the second method, a specially fabricated 'twin cage' was used for an uninterrupted breeding of these mosquitoes. This consisted of 2 cages, one of which had a dimension of 5' x 4' x 3' and the other was a fish tank (size 18" x 18" x 10") to which a rectangular metal frame was fixed and covered with plastic net. Both cages were connected together by a sleeve made of plastic net for easy movements of mosquitoes between the cages. After layering the bottom with sand, the fish tank was half filled with dechlorinated tap water. One of the advantages of this type of a cage set-up, consists of the study of the movement of mosquitoes between the breeding water source and the host. In this set-up a light beam across the passage, between the 2 cages with a photoelectric cell would 'measure' the periodic movement of mosquitoes between the breeding water source and the host as well as movements for swarming behaviour. Such a fabrication is being attempted. Eichornia speciosa, P. striatipes, C. axillaris, C. modesta, Ipomoea aquatica and Limnophila heterophylla were tried separately for larval attachment. When P. striatipes were not provided, evenly cut pieces of thermocol were allowed to float over the surface of water to facilitate oviposition.

In both methods, albino rabbit was used as host. Cotton-wool pad soaked in sugar solution was provided in the cages. To maintain high humidity, a requirement for the adult mosquitoes as indicated by Laurence et al the cages were placed over metal trays full of water and the sides of the cages were covered with damp lint. The larval diet consisted of a finely powdered mixture made with known proportions of dog biscuit (ca. 10 g), faecal pellets of rat (ca. 10 g), B-vitamins (ca. 0.5 to 0.8 g) and dried bovine liver (ca. 2.5 g) in 1000 ml of water. The pH of the culture medium was occasionally checked.

In addition to the above mentioned aquatic plants, alternative materials like thermocol, pith from Aeschynomene aspera and Cassava plants and commercially available cork were also tried for larval attachment. Petri culture methods was used to study the growth, moult and nutrient requirements of the larvae. For this, thermocol cut in circular shape, whose central portion had the form of a cup, was fitted tightly to a petri dish of size 50 x 70 mm. Dechlorinated tap water and larval food were poured into the depression up to the brim. The petri dish was covered after releasing the larvae and observations were made using a stereo-dissection microscope.

One of the most important problems associated with the laboratory rearing of Mansonia mosquitoes is the heavy mortality of the pre-imago stages, possibly resulting from either nutritional inadequacies or the decay of aquatic plants used in the culture. This has been indicated by Laurence et al. Though the larval diet supplemented with dried and powdered liver raised the nutritive value, as judged from the larval survival, the mortality remained high. Methods to improve diets are now being tried.

Among aquatic plants for larval attachment, P. striatipes, and E. speciosa are best preferred. However, these are not promising in the laboratory cultures as they decay very fast in the absence of sun light. This...
difficulty was overcome by the use of *C. axillaris*, *I. aquatica* and *C. nudiflora*. The former two gave very satisfactory results compared to *P. stratiotes* and *E. speciosa*, for they remained fresh for prolonged periods of time under the laboratory conditions.

Among the inert materials used for larval attachment, thermocol was the most suitable, though larval mortality remained high compared to those left on a live plant. Laurence *et al*3 made no comparison between larval mortality in the presence of "paper leaves" and "live plant". It is therefore difficult to judge the efficiency of thermocol as compared with "paper leaves". It is possible that though thermocol contains many air chambers, the difficulty in diffusion of carbon dioxide through the walls of the chambers could cause mortality.

In the present study, it was found that sugar is an inevitable factor for the survival of the adults and realisation of the full egg-laying potential. When these factors were satisfied, it is possible to get egg clusters regularly from the laboratory colonies of both *M. annulifera* and *M. uniformis*.

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APPEARANCE OF CHLORIDE CELLS IN THE GILLS OF TWO FRESHWATER TELEOSTS UNDER UREA STRESS

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Since the work of Keys1 the study of chloride ion secretion, through the gills of fishes, attracted the attention of a number of workers2–8. The cells of the gills involved in the chloride ion secretion were termed as chloride cells. There are other reports9–11 which show excretion of materials other than the chloride ions through these cells of some freshwater fishes. In the present study, it was observed that chloride cells developed on the gills of two freshwater teleosts *Channa punctatus* (Bl) and *Mystus (M.) vittatus* (Bl) under urea stress.

Thirty specimens of these two species of fishes were collected and acclimatised for 14 days under laboratory conditions. Each group was divided into two batches. One batch was put as control using tap water as the medium, whereas the remaining batches of *C. punctatus* and *M. (M.) vittatus* were kept in 18000 ppm12 and 11000 ppm of urea stress respectively. At these urea concentrations, no mortality was observed. The fishes from both the batches were sacrificed after 1 to 7 days. Simultaneously the fishes

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Figures 1–4.
1, 2. Gills of *C. punctatus* and *M. (M.) vittatus* respectively put as control. AgNO₃/HNO₃ × 1500.
3. Gill of *C. punctatus* put under 18000 ppm urea stress for 7 days. AgNO₃/HNO₃ × 1500. Note black depositions in the cells of (interlamellar) zone.
4. Gill of *M. (M.) vittatus* after 11000 ppm urea stress for 3 days. AgNO₃/HNO₃ × 1500. Cells of the basal portion of the secondary gill lamellae and interlamellar zone show positive response to chloride test (black deposit).