

PRACTICAL METHOD OF TRANSPORTING MOSQUITO EGGS

by

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Translated from Higijena (Yugoslavia), 2, 4, 319-321.

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The varieties of the *Anopheles* species of mosquito are today determined on the basis of the external appearance of the batches of their eggs, as there are no other reliable morphological signs for differentiating them.

The technique is to isolate the gravid females. Obtaining the eggs is a very simple matter, so that the work may be entrusted to non-technical personnel. The differentiation, however, can only be done by the experienced and well-trained eye of the expert.

For this reason the entomological investigation of this species is carried out at central establishments, where the necessary expert personnel is available, while the field anti-malarial units, each from its own territory, send the central laboratories the necessary material, either the adult mosquitos, or their eggs if the job is to examine *Anopheles* varieties.

The method most used in practice is to send the gravid females. However, the transport of the winged forms demands quite special means of shipping (shipping cage or shipping case), and always involves the risk of a good part of the mosquitos perishing en route. With the transport of eggs, on the contrary, this difficulty is eliminated. Shipping is simple, there is no loss en route, and what is more, when the specimens arrive for examination there is no need to proceed with the haste required when working with the live mosquitos.

We used this method, namely the shipping of eggs, in a study of *Anopheles* varieties and their distribution on the islands the Northern Adriatic, and it showed itself to be practical and reliable.

The shipping requisites are as follows: One or two wooden cases, holding glass tubes such as are used for shipping samples for bacteriological examination, a few sheets of filter-paper of medium thickness, and a 5% aqueous solution of formalin in a glass container.

From the filter-paper we cut out a sufficient number of discs of three kinds, about 1 mm less in diameter than the diameter of the glass tube. The discs of type "a" (see figure) have little tongue to them, type "b" are cut out into the shape

of a ring, type "c" are of ordinary disc shape.

The batches of eggs, as laid, are packed for shipment as follows. With a well-sharpened pencil of medium hardness, we write the batch-number on the tongue of an "a"-type disc. This number corresponds to a number in an accompanying list, which we later attach to the shipment and in which we give all necessary data relative to the batch. We pick up the paper disc with pincers, dip its edge into the formalin solution just enough so that all the surface will be moistened by capillarity, and put it on an object glass. Then we lay on it the cut-out bit of paper on which the female has laid the batch of eggs, and then with the pincers we put the disc carefully on the bottom of the glass shipping tube (after first covering the bottom with a thin layer of cotton wool, likewise moistened with formalin solution). With the disc properly in place, the little heap of eggs should be in the center, and the tongue of the disc should turn up and lie along the inner wall of the tube. On top of disc "a" we now lay discs "b" and "c" in turn, after moistening them in formalin in the manner described. Our batch of eggs is now enclosed in a little chamber of filter paper, protected against desiccation, putrefaction and break-up, and further ensured by the fact that the formalin kills the embryo and prevents the undesired emergence of the larva during transport. (See figure.)

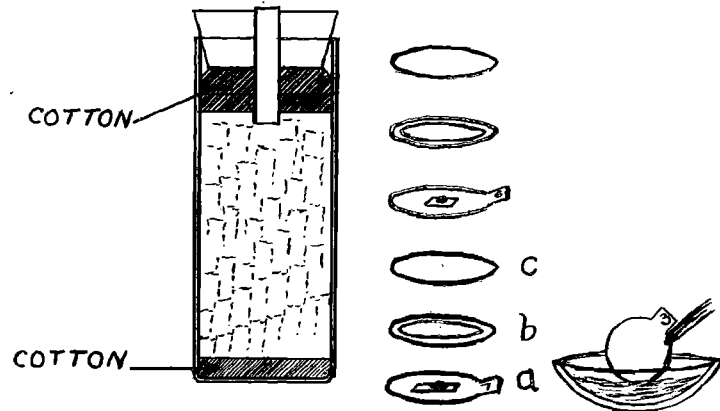
On top of this first batch we may put others, filling up the tube until there remains just enough room for a small wad of cotton and the stopper. Finally we secure the stopper with a strip of rubber tape, enclose the tube and the accompanying list in the wooden case, and all is ready for shipment. When it comes to unpacking the specimens, all that is necessary is to grasp the tongue of a disc with pincers and thus remove the batches one at a time from the tube.

The advantages of this method of ours of shipping the mosquito eggs are the simplicity of the technique, the good preservation of the material, and the small volume of the shipment as compared with its capacity, for fifty or more batches may be placed in a single tube.

Specimens will easily stand a journey of several days, because there will be no free liquid in the tube (thanks to the formalin-soaked discs), and the discs are neither too loosely or too tightly packed together.

To this method it might be objected that cutting out the discs is a job that takes a long time. However, if the method were more used, this difficulty could be obviated by having the discs manufactured.

We were already employing this method in 1937, when for a whole year we sent batches of Anopheles eggs from our seaboard regions to the Cantacuzène Institute in Bucarest. The eggs reached Professor Zotti, the parasitologist of that Institute, in perfect condition, and according to him were still usable weeks later for entomological studies.



(Redrawn from original)