# NOTES ON THE LABORATORY REARING OF SAND FLIES (DIPTERA: PSYCHODIDAE)

BRUCE F. ELDRIDGE, JOHN E. SCANLON[1] AND IAN M. ORENSTEIN
Department of Entomology, Walter Reed Army Institute of Research, Washington 12, D. C.

The role of phlebotomine sand flies as disseminators and transmitters of the sand fly fevers, the bartonelloses, and the leishmaniases, has long been recognized. Until recently, however, our knowledge of the biology of these interesting flies was quite scant. This situation partly is due to difficulties which have been encountered in rearing large enough numbers of flies for experimental purposes. The first colony of phlebotomine sand flies was established by Whittingham and Rook (1922) who maintained *P. papatasi* for three generations. Since that time various workers have employed a variety of techniques but all have been modifications to a greater or lesser degree of the technique described by Smith (1925). More recently, techniques somewhat different from those of Smith have been described by Unsworth and Gordon (1946) and Hertig and Johnson (1961). The techniques employed by all of these workers are elaborate and tend to be quite time-consuming.

The techniques which we are about to describe have gradually evolved from a composite of the techniques of several of the above workers. Some procedures have been entirely eliminated and many others greatly simplified. In spite of this streamlining, our colonies have remained at a very high level in terms of numbers of adults, and have supplied ample numbers of robust adults for experimental purposes. Modification of our techniques, however, may be needed when dealing with other species of sand flies.

Two species of *Phlebotomus* are being maintained in this laboratory. A colony of *P. papatasi* was established from wild-caught material collected in the Peshawar area of West Pakistan (Barnett and Suyemoto, 1961) and *P. argentipes* was subcolonized from Dr. Ghosh's colony at the Calcutta School of Tropical Medicine. While the two species came from markedly different climatic regions, a single rearing method has been satisfactory for both.

Adults are maintained in 1500-cubic-inch screened wood and plastic cages of a type commonly employed for mosquitoes. These cages are kept in a large insectary, at a temperature of 27° C. and a relative humidity of approximately 60 percent. As a carbohydrate source, we provide 5 percent dextrose and fresh sliced apples. Although it has been reported sand flies are extremely sensitive to bacterial contamination, we do not employ sterile techniques in handling of these materials. No deleterious effects on the sand flies have ever been observed because of this, however.

As a blood-meal source, we use immobilized, shaved chicks in the case of *P. papatasi* and immobilized, shaved guinea pigs in the case of *P. argentipes*. Either animal is satisfactory for both sand fly species, but this arrangement has yielded maximum egg production in our colonies. *P. argentipes* in our laboratory will feed only in total darkness, while *P. papatasi* will feed under almost any lighting conditions. After two hours of exposure to a guinea pig in darkness, more than 60 percent of the *P. argentipes* will have taken a blood meal. A somewhat longer exposure is required for an equal percentage of *P. papatasi* to feed. When the animals are left in the cages overnight, nearly all of the females of either species take a blood meal.

After 48 hours have elapsed from the time of the removal of the blood-source animal, gravid females are placed in

[1] Present address: SEATO Medical Research Laboratory, Bangkok, Thailand.

10 x 1.5 cm. test tubes, two females to a tube. The tubes are then cotton-stoppered, and inverted in a 250 ml. beaker in which a moist gauze sponge has been placed. The beakers are placed in incubators maintained at a temperature of 28° C. The incubators we use are commercially converted household refrigerators. Beakers for oviposition can be held in the bottle racks in the door, and the larval containers (described later) in the main compartment. Since the overwhelming majority of females usually take a blood meal, no attempt is made to distinguish gravid from non-gravid females.

Generally oviposition occurs in about 85 percent of the tubes. We originally placed single females in 7.5 x 1 cm. test tubes. Although there is no difference in the number of eggs produced per female, we find it more convenient to use two females in the larger tube. The number of eggs produced per female by *P. argentipes* when placed two per tube is $47.07 \pm 16.75$ (mean of 30 sand flies $\pm$ standard error). Under these same conditions, the number produced by *P. papatasi* is $40.39 \pm 16.19$. We have found that deviation from these combinations of tube sizes and number of sand flies results in a decrease in egg production. Apparently, oviposition is encouraged by close confinement of the females, but overcrowding of them results in competition for oviposition space and a resultant decrease in egg production. Eggs are usually deposited upon the cotton plug within 24 to 48 hours after confinement of the females.

When oviposition is complete, the eggs are flushed with water into larval rearing containers. These larval rearing containers are unglazed ceramic pots 3 inches in diameter and 3 inches in height. They are obtained from a local ceramic art dealer. Other types of unglazed ceramic containers have been found equally satisfactory. The principle requirement for a satisfactory container is a porosity which permits drainage of the flushing water, but retains sufficient moisture to prevent drying of the larval rearing media. A suitable larval density is obtained by placing the eggs from 20–30 tubes (i.e. from 40–60 females) in each pot, but this figure can be deviated from without adverse affect. The pots containing eggs are placed upon stacks of paper towels to facilitate drainage, which usually takes 24 to 48 hours. When the flushing water has drained, a muslin cover is fastened to each pot with a rubber band. The pots are then placed in small pans lined with damp towels, 5 pots to a pan. The towels are folded to several thicknesses. From this time until adult emergence, the pans are held in the incubators at a temperature of 28° C. and approximately 80 per cent relative humidity.

The eggs of both species hatch 8 days from the time of oviposition. At this time, a very small amount of ground rabbit feces is sprinkled on the bottom of the rearing pot. The desirability of avoiding overfeeding of the larvae will be discussed subsequently. We check each pot three times a week at which time we add food if needed and re-dampen the towels upon which the pots are setting. These two operations require the greatest amount of experience and judgment of any of the rearing techniques. It is not possible to prescribe an exact quantity of food to be given, nor an exact amount of dampening of the towels. Generally speaking, we cover the surface of the larval medium with a thin layer of fresh food each time and thoroughly wet the towels. The appearance of each pot guides us in determining the amount of food and moisture required.

Approximately 35 days after oviposition in the case of *P. argentipes* and 45 days in the case of *P. papatasi,* adult emergence begins. At this time, adult sand flies are released into the aforementioned cages and the life cycle is complete. Emergence is completed in about a week in the case of *P. argentipes,* but is strung out to almost 2 weeks with *P. papatasi.* In our laboratory, adult female sand flies survive up to 3 weeks, if they are not used for egg production.

Larval food is prepared in the following

manner: rabbit feces are first autoclaved, then dried in an oven for 24 hours. After grinding in a commercial food grinder, the food is ready for use.

Some workers have encountered difficulties arising from mites and fungi contaminating the larval pots. In our laboratory, we found heavy mite and fungus infestations to be definitely associated with overfeeding, especially in the earlier larval stages. We formerly took elaborate precautions to prevent such infestations, but our efforts were largely unsuccessful, despite the use of practically aseptic techniques. We have now abandoned all such precautions including even the autoclaving of pots between generations. We simply tap out the old rabbit feces (which are autoclaved) and wipe the pot out with a paper towel. We have found that this not only saves time, but provides a residuum of food for the freshly hatched larvae. Although we still have mites and fungi to varying degrees, as long as the amount of food in the pots does not become excessive, we are not troubled by them in any way.

Although we have eliminated many unnecessary procedures from our rearing techniques with a corresponding savings of time and effort, there probably are still a number of short cuts which can be taken. In spite of our comparatively casual approach to sand fly rearing we have not experienced any diminishment of colony vigor, or diapause in the three years we have been maintaining the colonies.

*References Cited*

BARNETT, H. C., and SUYEMOTO, W. 1961. Field studies on sandfly fever and kala-azar in Pakistan, in Iran, and in Baltistan (Little Tibet) Kashmir. Trans. N. Y. Acad. Sci. 23:609–617.

HERTIG, M., and JOHNSON, P. T. 1961. The rearing of *Phlebotomus* sandflies (Diptera: Psychodidae). I. Technique. Ann. Ent. Soc. Amer. 54:753–764.

SMITH, R. O. A. 1925. A note on a simple method of breeding sandflies. Ind. Jour. Med. Res. 12:741–742.

UNSWORTH, K., and GORDON, R. M. 1946. The maintenance of a colony of *Phlebotomus papatasii* in Great Britain. Ann. Trop. Med. Parasit. 40:219–227.

WHITTINGHAM, H. E., and ROOK, A. F. 1922. Demonstration of the life cycle of *Phlebotomus papatasii* and its maintenance in captivity. Trans. Roy. Soc. Trop. Med. and Hyg. 16:262–266.

## CDC MOSQUITO CONTROL COURSE

The Communicable Disease Center of the U. S. Department of Health, Education, and Welfare, Public Health Service announces a course in mosquito control (Course No. 231) to be given November 4–8, 1963 by the Training Branch at the Center in Atlanta. As usual, the Director will be Dr. Harry D. Pratt. The announcement lists as "Special Guest Lecturer: Mr. R. E. Dorer, Past President of the American Mosquito Control Association, and Chief, Bureau of Insect and Rodent Control, Virginia State Department of Health."

The purpose of the course is "To provide public health personnel with a basic understanding of the control of mosquitoes affecting the health and well-being of man and the control of mosquito-borne diseases. Primary emphasis is placed on mosquito identification and biology, and field work where the trainees have time to practice control procedures developed in the classroom and laboratory."

For application forms and additional information address: Communicable Disease Center, Atlanta 22, Georgia, Attention: Chief, Training Branch.