

# Laboratory Culture of two *Phlebotomus* Species, *P. papatasi* and *P. orientalis* \*

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Laboratory colonies of two *Phlebotomus* species, *P. papatasi* and *P. orientalis*, are currently being maintained at the United States Naval Medical Research Unit No. 3, Cairo, UAR. Established in 1959 and 1962 respectively, the colonies have yielded more than a million sandflies for use in studies of phlebotomus fever virus-vector relationships, kala-azar vector potentialities, and susceptibility to insecticides. This report contains a brief description of the rearing techniques employed.

## ENVIRONMENTAL CONDITIONS

### *Temperature and humidity*

An ambient temperature of 80° F-85° F (26.5° C-29.5° C) is maintained, with an ambient relative humidity of 75%-85%.

### *Rearing containers*

These are unglazed clay pots (approximately four inches in diameter and three inches high—10 × 7.5 cm) lined with plaster of Paris. The mouth is covered with muslin or fine-meshed nylon netting.

### *Moisture beds*

The moisture beds for the rearing containers are coated galvanized metal trays, 18 inches × 18 inches × 2 inches high (45 × 45 × 5 cm), containing a one-inch (2.5 cm) layer of sand and a half-inch (1.3 cm) layer of absorbent cotton. Sand and cotton are moistened *ad libitum* short of complete saturation.

### *Holding cages for adults*

These are eight-inch cubes (20 × 20 × 20 cm), wooden-framed. The top and two sides are of glass, and the bottom of wood; one side is composed of a

fine-mesh wire screen and the other side is provided with a sleeve.

### *Food*

Larvae are given a dry pulverized mixture of rabbit faeces and ovine or bovine blood. Adults (females) receive guinea-pig blood: adults of both sexes are given raisins boiled in salt water.

### *Mould control*

To control mould in the rearing containers the larval food is covered with a quarter-inch (0.6 cm) layer of sand-soil mixture. The containers are sprayed twice weekly with aqueous cycloheximide solution (2000 units/ml).

## REARING PROCEDURE

Mated females are distributed, 10 to a rearing container, for egg-laying. Dead females are removed daily to minimize mould growth, and dates of first oviposition are marked on the containers. Food is added when larvae begin to hatch (seven to eight days after oviposition) and is replenished as depleted. Containers are examined twice weekly to ensure proper moisture conditions, i.e., food and container walls moist but not waterlogged. If necessary, excessive mould is removed and the containers are sprayed with fungicide. At the time of pupation accumulated larva faeces are removed by sifting through a fine mesh screen. As adults emerge they are aspirated from the containers and held in a cage for two to three days for mating. Females are then allowed to feed on the shaved abdomens of guinea-pigs and are distributed to rearing containers to initiate the next generation. Males are discarded after mating.

Major factors that contribute to successful colonization are (1) maintenance of sufficient moisture to facilitate egg-laying and to promote larval development, and (2) controlling growth of mould, which immobilizes larvae and reduces their feeding activity. Additional factors particularly applicable to the rearing of *P. orientalis* are (1) feeding of adults at the proper age (two to five days after emergence) and

\* The opinions and assertions contained in this paper are those of the author and are not to be construed as official or reflecting the views of the United States Navy Department or the naval service at large.

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(2) use of great care in handling adults, which are more fragile than the larger *P. papatasi* and more prone to succumb to rough manipulation.

The adult-to-adult generation time for sandflies reared under the above conditions is six to seven weeks. This period can be shortened to four to five weeks by raising the rearing temperature to 90° F (32° C), but the longevity of adult flies is reduced concomitantly. Larval development is markedly retarded by copious mould growth or excessive moisture, and the first instars experience the highest mortality rate. During winter months, despite the constant 80° F-85° F (26.5° C-29.5° C) temperature of the rearing chamber, up to 5% of the larvae hibernate in the fourth instar. Pupation may be stimulated by transferring larvae to clean containers. Each mated female may be expected to yield an average of 10 adult female progeny.

#### *Effect of diet variation*

Adult *P. papatasi* can be fed on any of a variety of animal bloods; human, guinea-pig, rabbit, mouse, chick and lizard bloods have been used with equal success. In fact the majority of Egyptian specimens do not require a blood meal for oviposition; one line has been reared autogenously through 14 generations. Flies given access to blood, however, yield more progeny. Experience with *P. orientalis* is limited to the use of human and guinea-pig blood. This species has not been observed to lay eggs without a previous blood meal. Adults are routinely fed on living hosts but may be successfully fed on heparinized whole blood through animal membranes, i.e., plucked mouse skin or baudruche (gold-beater's skin). During membrane feedings, the sandflies and blood meal are held at 80° F (26.5° C).

Blood is included in the larval diet to provide additional organic nutrient. Preliminary evidence suggests that it may be omitted without deleterious effect on larval development and controlled experiments which substantiate this fact are in progress.

Because complex organic nutrients from natural sources have proved adequate for rearing larvae and adults, no attempt has been made to develop stan-

dard chemically-defined synthetic or semi-synthetic diets.

#### *Mating habits*

It has not been found necessary to devise special conditions to induce mating. Both species mate under diverse environmental circumstances, e.g., temperature range from 75° F to 90° F (24° C to 32° C), 20%-95% relative humidity, in small rearing containers or large holding cages, and in bright light or total darkness. The minimum mating age is about 12 hours and fertilization may occur before or after feeding.

#### *Parasite control*

The mites which parasitize wild *P. papatasi*, although not removed from the specimens used to establish the colony, do not thrive under colony conditions and do not constitute a hazard to colonization. Free-living mites which compete with sandfly larvae for food, and ants which attack larvae, pupae and adults are excluded from the colony by liquid-filled moats. Larval food is autoclaved as a further precaution against the introduction of predatory arthropods.

### EFFECTS OF COLONIZATION

The influence of prolonged colonization on the morphology and physiology of sandflies has not been studied systematically. However, the following observations have been made during the course of our work.

(a) Though not yet quantified, the size of colony flies appears slightly larger than that of wild-caught specimens.

(b) *P. papatasi* exhibits a slight but consistent increase in resistance to DDT and dieldrin between the first and fifth laboratory generations. This may be a manifestation of so-called "vigour tolerance".

(c) First through twentieth generation *P. papatasi* exhibit the same degree of susceptibility to sandfly fever virus as wild-caught specimens.