*Bull. Org. mond. Santé*
*Bull. Wld Hlth Org.* } 1964, **31**, 569-570



# Laboratory Colonization of Central American *Phlebotomus* Sandflies

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The laboratory rearing of a considerable number of species of *Phlebotomus* sandflies has now been carried out in both hemispheres. The degree of success, however, varies with the species. Some are easy to rear and carry on in successive laboratory generations. Others can be easily carried from eggs laid by wild-caught females through the immature stages to the adult, but the reared adults may feed with great reluctance in the laboratory on any animal, even their normal hosts. Still other species are difficult to carry beyond the first larval stage. In practically all cases, however, even those species easiest to rear require the nearly constant attention of a skilled technician.

In starting work with a sandfly which has not been reared previously, one must be prepared to modify or work out *de novo* the technique appropriate for that species. Our own experience will illustrate this point. The writer had previously reared *Phlebotomus* in China, the USA and Peru by a technique which during that 30-year period became more or less standard. In rearing Panamanian sandflies the "standard" technique had to be modified at practically every step; e.g., the size of the containers for holding individuals and securing eggs; the larval food; a difference of a few degrees of temperature was found to be critical, etc. A team of investigators who had spent some time in our laboratory met with indifferent success in attempting to rear some of the same species in another Central American country. One of our own staff members, thoroughly experienced and skilled in rearing Panamanian sandflies, was successful, in later work in Egypt, with certain species but we understand that experimental work with one potential leishmaniasis vector is still hampered by the difficulty of rearing it.

We have recently reviewed sandfly rearing techniques and described in detail our present methods of rearing Panamanian *Phlebotomus* and the behaviour of individual species in culture (Hertig & Johnson, 1961; Johnson & Hertig, 1961). For the purposes of this report it seems unnecessary to go into the technique except to point out that the unit breeding-vessel is a small porous pot in which the breeding of one or two hundred sandflies may often be carried through. In the tropics the various hazards, such as mites, ants and fungi, make it desirable or even mandatory to have the breeding-units small enough so that they may be satisfactorily protected by moats and so that they may be readily inspected under the dissecting microscope. One or two species in Europe and South America have been reared in mass colonies in large structures designed for the purpose. In concentrating so many eggs in one basket, however, the risk would be correspondingly great. While larger breeding units than our small pots may be devised (we have had some success with larger units), the wholesale and continuous production of vast numbers of tropical sandflies, as for a biological control project, would constitute in itself a project of some magnitude which would have to be worked out.

In connexion with any *Phlebotomus* control project, biological or otherwise, the following considerations may be noted: DDT and comparable residual insecticides have been shown to provide effective control in urban and village areas, i.e., in more or less compact communities. (Sandfly control is a common "by-product" of malaria control in the Old World.) Isolated dwellings, forests and open country present different problems, usually with no known feasible solutions. In the New World the most widespread sandfly-borne disease, American cutaneous leishmaniasis, is endemic in vast, sparsely-inhabited forests. A biological control project seems out of the question. Treatment of individual dwellings and their immediate surroundings with DDT may provide some protection. In Peru, bartonellosis and a local strain of cutaneous leishmaniasis are endemic in sparsely-inhabited areas with sparse vegetation. Biological control again seems out of the question but hydroelectric con-

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struction camps and installations in the endemic zone have been successfully protected by DDT for a number of years against both diseases.

## VARIABLE FACTORS IN COLONIZATION

### *Containers and food*

Our standard breeding-vessel, a porous pot lined with plaster of Paris, and with a cloth cover, provides: (*a*) a substrate for which the moisture can be regulated (wetness must be avoided); (*b*) high relative humidity; (*c*) a convenient means of confining the emerging adults (and excluding other insects). Any type or size of container which provides these essentials could probably be made the basis for a rearing system, the details of which would have to be tested or worked out for each species.

The bare plaster of the breeding pot, plus the accumulation of food material, provides a convenient and satisfactory physical medium. A variety of other substrates has been used or tried out, mostly involving soil. The latter would probably be desirable in any attempt at mass colonization. We successfully reared several species in small terraria containing soil covered with a layer of forest leaves, simulating one of the local natural habitats.

The suitability of larval food must be determined for each species. The larvae are scavengers in nature. Faeces, usually of laboratory rodents, have been the basis of most larval diets. For certain species we have found it necessary to add partly-rotted forest leaves, and in general have found it desirable to enrich the food with cut-up insect bodies.

### *Live animals as hosts*

The blood meal of the adults can be provided only by its feeding on an animal. Many species feed readily on a variety of laboratory animals. Guinea-pigs are eminently suitable. However, some species are reluctant to feed in the laboratory, regardless of host, time of day, or temperature. In such cases breeding stock must be maintained by eggs from wild-caught females.

### *Genetic variability*

A small proportion (about 5% of wild-caught females) of one Panamanian species, *Phlebotomus gomezi*, can give rise to successive autogenous laboratory generations (Johnson, 1961). Autogenous and "normal" strains are genetically distinct. We have no other information about genetic variability. We would expect, however, to find that widely-separated geographic strains of the same species actually differ in various ways.

### *Mating*

This has never been a problem in *Phlebotomus* rearing comparable to that encountered with some mosquitos. Mating apparently takes place in rearing-vessels and releasing-cages either before or after a blood meal. It is desirable, and in some cases probably necessary, to have males constantly present.

### *Parasites*

The whole genus *Phlebotomus* is relatively free of parasites. While a variety of parasitic infections is known, they seem to have little or no deleterious effect. This is true of the natural infections of man-biting species with leptomonad flagellates (Johnson, McConnell & Hertig, 1963) and of rather heavy trypanosome infections of a few other Panamanian sandflies (McConnell & Correa, 1964). None of the recognizable "natural" parasites has ever been carried over into laboratory generations. Bacteria and fungi, probably the result of conditions in the laboratory containers, may be troublesome.

### *Avoiding disease*

Our one serious problem has been a species of *Aspergillus*, original source unknown, which attacks and kills larvae. At one time nystatin helped to keep this fungus in check. We now remove the pupae, wash them several times in tap-water plus antibiotics, and transfer them to a fresh pot. This procedure, combined with the routine autoclaving of containers and food and the use of sterile instruments, usually holds the *Aspergillus* to manageable levels. Mites, and at times ants, can be serious pests. A water moat is generally effective in preventing their entrance or spread.

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