

## RESEARCH NOTE

## A New Larval Diet for Colonization of Phlebotominae Sand Flies

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Key words: sand flies - *Lutzomyia longipalpis* - larval food

Phlebotominae sand fly colonies are very important for studies of vector biology and the interaction of sand flies and *Leishmania*. Although, it requires excessive care, especially for immature stages, some sand flies species have been colonized in large numbers [R Killick-Kendrick et al. 1991 *Parassitologia* 33 (Suppl.1): 321-333]. The larval diet is an important part of successful laboratory breeding due to the often excessive larval mortality caused by improper food.

MP Barretto (1942 Thesis Faculdade de Medicina USP, 162 pp.) suggested that during the immature stages period, the feeding preferences of sand flies from the New World may vary according to geographical location. However, the larval stage can use any kind of food, in situations where their preferred food sources are not available.

IA Sherlock and VA Sherlock (1959 *Rev Brasil Biol* 19: 229-250) made some experiments with *Lutzomyia longipalpis* in order to determine the most adequate culture conditions and medium for feeding immature stages. In these experiments decomposed organic vegetable matter products or dry bovine feces were the most suitable for larval development.

T Gemetchu (1971 *Trans R Soc Trop Med Hyg* 65: 682-684) tested liver powder as an alimentary

diet of *Phlebotomus longipes* larvae. He observed that this feeding regime was better than a mixture of rabbit feces, and suggested that the duration of the larval period varied according to the composition of the diet. The liver powder as larval diet was used by K Killick-Kendrick et al. (1977 *J Med Ent* 13: 429-440) and R Ward (*Med Entomol* 14: 469-476) too, for breeding of New World sand flies.

*L. cruciata*, *L. anthophora*, *L. shannoni* and *L. vexator* were reared in laboratory using the mixture of rabbit feces and laboratory rabbit chow as a feeding regime for larvae stages development. It was observed that larval mortality due to excessive fungal growth was reduced by ageing (or allowing the fermentation of the food) (DG Young et al. 1981 *J Med Entomol* 18: 446). After that, this larval food has been used in many laboratory colonies successfully (RG Endris et al. 1982 *Mosquitos News* 42: 400-407, R Killick-Kendrick & M Killick-Kendrick 1987 *Ann Parasitol Hum Comp* 6: 354-356, Killick-Kendrick et al. *loc. cit.*). B Modi and R Tesh (*J Med Entomol* 20: 568-569) used a modification of this mentioned larval diet. They added beef liver powder to the mixture of dried rabbit feces and rabbit chow.

In order to improve and optimize conditions for sand flies developing in laboratory colonies, we tested a new appropriate diet for immature stages. The sand flies were taken from our *L. longipalpis* laboratory colony, maintained according to ED Wermelinger et al. (1987 *Mem Inst Oswaldo Cruz* 82: 441-442), with the following modifications: after a blood meal, batches of 40 females were placed in rearing pots for oviposition and they were fed on a saturated sucrose solution according to NA Souza et al. (1995 *Mem Inst Oswaldo Cruz* 90: 751-757). The pots were observed daily and dead females were removed. The larval food was offered between 8 and 10 days after the transportation of engorged females to rearing pots.

*Test diet* - The larval food was prepared using a mixture of liver powder (Integalmédica, BZ Ind.), amino acids (histidine, isoleucine, cystine, methionine, tyrosine, cystine, leucine, glycine, proline, alanine, lysine, serine, valine - Probiótica, BZ Ind.), fish food, (Vitormônio, BZ Ind, E Rangel et al. 1985 *Mem Inst Oswaldo Cruz* 80: 219-226) and pollen grains (proteins, glycidic lipids, potassium, phosphorous, calcium, iron, vitamin B1, B2, C and B-carotene - Herbarium, BZ Ind.). Five grams of each component were used. After grinding, the components were mixed, sifted and distributed into small-sterilized glass tubes. For the third and fourth larval instars, the food was ready to be consumed. However, in the case of the first and the

second instars, the larval food was sifted through a piece of nylon, before use so that we could obtain a very thin powder, since young larva have limited activity and they move slowly in the culture medium. In these experiments of 1,000 eggs each were treated by offering the test larval diet. In control batches fish food powder (Vitormonio) was used as diet, applied according to our breeding methodology (EF Rangel et al. 1986 *Mem Inst Oswaldo Cruz* 81: 431-438). The new larval food was tested for five consecutive life cycles with three repetitions each.

The obtained results revealed this food to be a satisfactory larval diet. Moreover, when compared with fish food the level of fungal contamination was greatly reduced, larval mortality decreased and output increased in relation to the number of adults emerged/number of eggs observed during five life cycles: 50.9, 38.3, 53.7, 52.3 and 48.8%. When fish food was used, the percentages of adults emerged were 24.9, 28.4, 20.4, 27.3 and 27.6% (Fig. 1). Significant differences were observed in terms of the duration of life cycle from eggs to adults, when the two larval diet were compared: (1) fish food: mean -

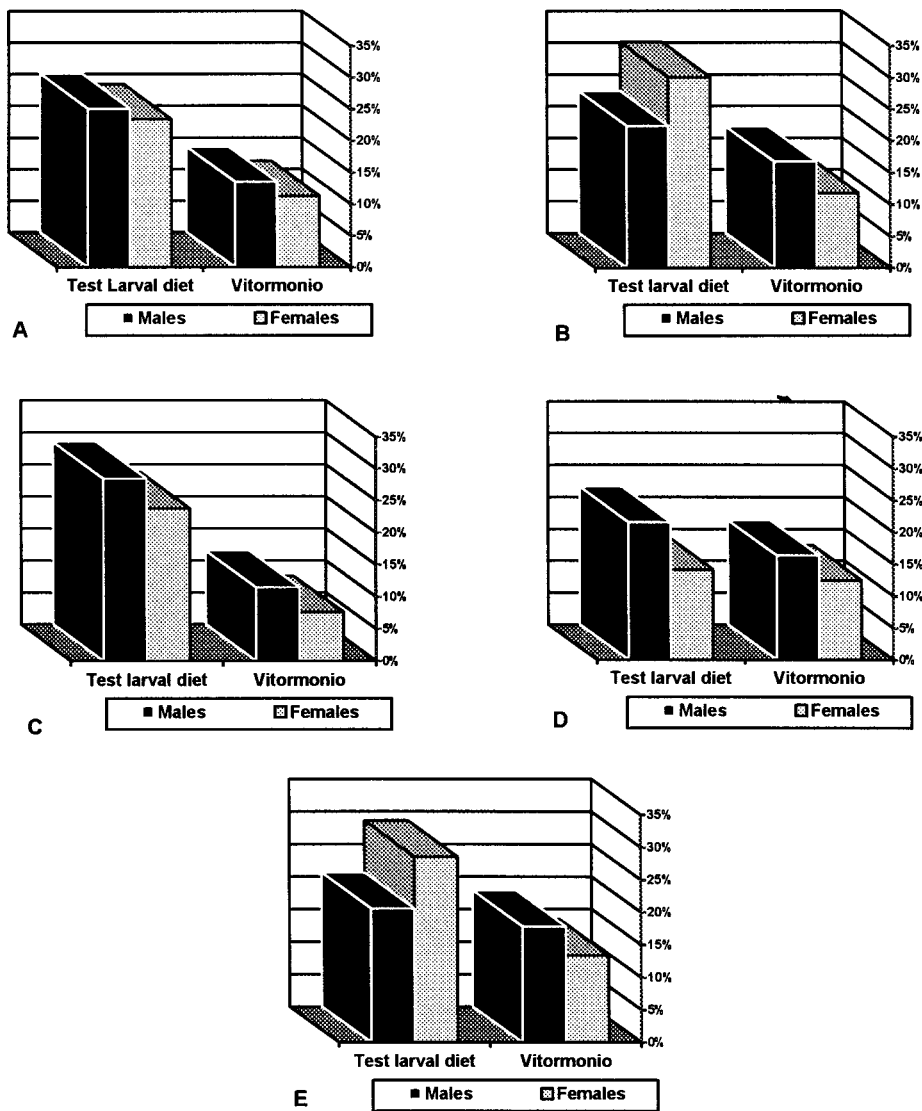


Fig. 1: development of the colony of *Lutzomyia longipalpis* comparing the use of two kinds of larval food. A: 1st life cycle; B: 2nd life cycle; C: 3rd life cycle; D: 4th life cycle; E: 5th life cycle.

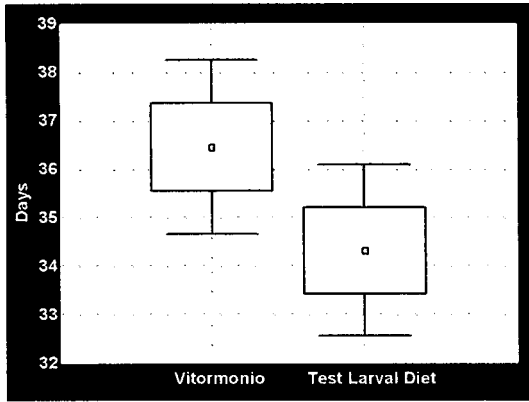


Fig. 2: duration of cycle from eggs to adults. *Lutzomyia longipalpis*. (□- mean, □+/- 1.0 std.dev., I +/- 1.96 std. dev.)

36.5, std. dev. 0.92 and (2) test larval diet: mean - 34.3, std. dev. 0.90 (Fig. 2). The good results obtained using this larval diet for *L. longipalpis* colony were observed with other Neotropical sand flies, such as *L. intermedia* and *L. whitmani*.

Souza et al. (*loc. cit.*) suggested that mix of sugars added to a source of amino acids could be the ideal diet for adult sand flies. We are of the opinion that its use combined with the new diet reported in this study and a source of amino acids could result in improved maintenance of sand fly colonies in the laboratory.

*Acknowledgments:* to Dr Gabriel Grimaldi Jr, Instituto Oswaldo Cruz, for contribution in the revision of the text and Prof. João Carlos Peixoto, Federal University of Rio de Janeiro, for the assistance with graphical illustration.