## PHORETIC RELATIONSHIP BETWEEN BIRD MALLOPHAGA AND MOSQUITOES

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The phoretic relationship between Mallophaga and Hippoboscidae is common. Keirans (1975a) listed over 400 records of biting lice attached to louse flies seeking transfer from one vertebrate host to another. Phoresy appears much less common between Mallophaga and other insects. Keirans (1975b) cited records of biting lice from Siphonaptera, Diptera (other than Hippoboscidae), Odonata, Hymenoptera and Lepidoptera. The few records of Mallophaga from mosquitoes known to us involve the mammalian parasite, *Bovicola meyeri*, and *Aedes* spp.

We report here 2 separate recoveries of bird Mallophaga associated with *Culex* mosquitoes from Ecuador and 1 from Brazil. During the period 1974–1978, more than 500,000 mosquitoes have been collected and processed for virus isolations in cooperative arbovirus studies conducted in this laboratory and by Dr. E. Gutierrez, National Institute of Hygiene, Guayaquil, Ecuador. The mosquitoes have been primarily collected in CDC light traps, supplemented with carbon dioxide.

A biting louse (*Formiphagus* sp.) was found attached by its mouth parts to the proboscis of a ♀ *Cx. nigripalpus* mosquito collected in Los Rios Province, Ecuador, June 11, 1974. Over 170,000 *Cx. nigripalpus* have been examined from Ecuador. A second biting louse (*Formicaricola* sp.) was recovered in association with a pool of *Cx. vomerifer* collected in Vinces Province, Ecuador, May 10, 1978. At first glance the louse appeared to be affixed to a leg, but it came free so readily there may have been another attachment site. Approximately 1,000 *Cx. vomerifer* have been examined from Ecuador.

The third recovery of a mallophagan from a mosquito involved a louse (*Formiphagus* sp.) attached to the proboscis of a ♀ *Culex (Culex)* sp. collected in a CDC light trap (with carbon dioxide) in Iguape, São Paulo State, Brazil, April 19, 1976. The mosquito was too battered to permit specific identification. Approximately 40,000 mosquitoes (2200 of them *Culex (Culex)* spp.) were collected by a variety of methods in 1975–76 in São Paulo State by Dr. Oscar de Souza Lopes, Instituto Adolfo Lutz, São Paulo. The mosquitoes were received by this laboratory for virus testing in connection with an epidemic presumably due to a newly recognized flavivirus, Rocio virus.

We thank Dr. K. C. Emerson, the distinguished authority on Mallophaga, U.S. National Museum, Washington, D. C., for identifying the lice. Since all were ♀♀, specific determinations were not possible. He has informed us that the hosts of these 2 genera are antbirds, family Formicaridae, found only in the Neotropics.

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## A RAPID GONOTROPHIC CYCLE IN *CHAGASIA BONNEAE* FROM BRAZIL

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During a study of *Anopheles darlingi* Root in Brazil (Charlwood & Wilkes 1978) several *Chagasia bonneae* Root were caught biting man at dusk and were subsequently dissected using Polovodova's technique for physiological age grading of blood sucking insects (Detinova 1962). The results may be of interest in future epidemiological investigations involving this little studied species.

The study took place in April 1978 at the end of the rainy season in the village of Aripuaná (10° 19′ 42″ S 59° 12′ 30″ W population ca. 600) in the northern part of the state of Matto Grosso, Brazil. Biting catches were performed approximately 25 m. from the tropical rain forest which surrounds the village. A collector, sitting under a temporary shelter, caught mosquitoes in test tubes as they came to

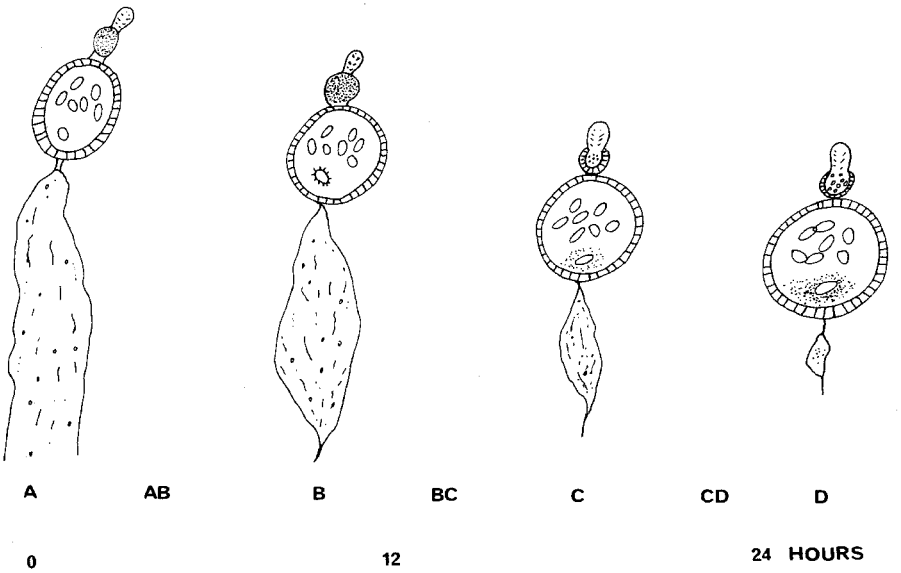


Figure 1. Appearance of sac stages in *Chagasia bonneae* and the estimated time from the last oviposition. (After Detinova 1962).

feed on his exposed lower legs. The first arrivals were dissected each evening and the remainder were dissected the following morning. Dissections were performed in the manner described by Gillies and Wilkes (1965) and during dissection the state of the follicular stalks was noted. In parous flies the size of any sac left by a previous egg was subjectively graded as being 'a,' 'ab,' 'b,' etc., Fig. I (Detinova 1962) and in this way the time since the last oviposition was estimated.

Results, Table I, show that there was a relatively even age distribution, with the oldest insects dissected being three parous. Almost all of the parous mosquitoes (84.5%) had either 'a,' 'ab,' or 'b' sacs (Table 2). Assuming that sac contraction occurs at the same rate in this species as it does in *An. gambiae* Giles and *An. funestus* Giles, in that it takes 12 hr or less to reach stage 'c' (Gillies & Wilkes 1965), these results imply that the *Ch. bonneae* females were coming to feed on man a short time (within 12 hr) after oviposition. The few dissections performed on blood-fed individuals showed that the ovaries were fully mature within 48 hr after feeding. If oviposition occurs without delay, these results show that this mosquito has a gonotrophic cycle, from one blood meal to the next, of the order of just 2 days. This rapid gonotrophic cycle will increase the frequency of biting in comparison to a species that has an obligatory resting period after oviposition.

Table 1. Results of age-grading dissections of *Chagasia bonneae*

| | Number of dilatations | | |
|---|---|---|---|
| Nulliparous | 1 | 2 | 3 |
| 44 | 21 | 14 | 3 |

Table 2. Sac stages of parous *C. bonneae* dissected

| Sac Stage | a | ab | b | bc | c | cd | d | No Sac |
|---|---|---|---|---|---|---|---|---|
| | 6 | 19 | 7 | 3 | 0 | 1 | 0 | 1 |
| % | 16 | 51 | 19 | 8 | 0 | 3 | 0 | 3 |

Were this species capable of transmitting disease, this feeding pattern would make this mosquito a most efficient vector.

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## SEPARATION OF AEDINE EGGS FROM SOIL SAMPLE DEBRIS USING HYDROGEN PEROXIDE

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The collection and analysis of soil samples from areas subject to transient inundation is a survey technique important to mosquito abatement districts where floodwater mosquitoes are a major problem. Samples collected in late autumn are of value in determining intensity and distribution of the succeeding year's spring generation. Summer collections are useful in determining site distribution of eggs prior and subsequent to inundation.

The Illinois Egg Separation Procedure described by Horsfall (1956) is adequate for separating eggs from inorganic and most organic materials in a sample. Terminal separation of eggs from minute, organic debris in water is currently done optically and is time consuming if many eggs are present.

A method developed at the Macon Mosquito Abatement District has greatly reduced the time.

METHODS AND MATERIALS. The new method of terminal separation follows the Illinois Egg Separation Procedure. In that procedure, eggs are separated from materials of dissimilar density by flotation in saturated solutions of NaCl in water and in water alone. The final result is a mixture of eggs and organic debris of similar density in the bottom of a clean casserole of water. The material within the casserole is viewed through a stereomicroscope and eggs are removed by pipette from surrounding debris.

The new method changes the optical separation procedure in three ways: (1) separation takes place in a casserole lined with paraffin wax, (2) the mixture of eggs and debris is heated prior to separation, and (3) a hydrogen peroxide solution in water is used instead of water alone.

Separation is made in a 150 ml porcelain casserole in which chips of paraffin wax have been melted and allowed to harden into a layer on the bottom. Bubbles may form on the bottom of a casserole during separation if not lined with wax. Wax should cover only the bottom of the casserole because wax on the sides will impede egg recovery. A wax lined casserole may be used for repeated separations.

The eggs and debris are heated by decanting water from the clean casserole containing the mixture and adding approximately 100 mls of 60°C to 70°C water. After several seconds the contents of the casserole are poured through a 100 mesh screen, trapping the eggs and debris. The heat treated mixture is washed with minimal water into the wax lined casserole.

The hydrogen peroxide solution is prepared by diluting commercially available 3% hydrogen peroxide solution with water. A 0.3% solution will usually give satisfactory separation, however, more concentrated solutions may be used. The solution is used while it is at room temperature (25°C). Solutions have been stored for 24 hr at room temperature without deterioration.

Approximately 100 ml of the diluted hydrogen peroxide solution is added to the wax-lined casserole containing the heat treated mixture. The action of hydrogen peroxide results in bubble formation within debris particles which are floated to the surface. Aedine eggs are not affected in this way. The contents of the casserole are gently spun to center the eggs on the wax and dislodge bubbles which may float some eggs. Eggs are pipetted from the