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# Experimental Observation of Developmental Period of *Onchocerca volvulus* in Black Fly, *Simulium ochraceum*

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The 3 species of black flies, *Simulium ochraceum*, *S. metallicum* and *S. callidum*, are highly anthropophilic in endemic areas of human onchocerciasis in Guatemala. Of the three, *S. ochraceum* is considered the most important vector of the disease, followed by *S. metallicum* and *S. callidum* (Omar and Garms, 1975; Collins, 1979). De Leon and Duke (1966) showed that development of the infective larvae of *Onchocerca volvulus* was complete by the 7th or 8th days in *S. ochraceum* within the temperature range of 22–27 C. Collins *et al.* (1977) also showed that the 3rd stage larvae were first seen on the 8th day at 20–26 C. Because of these wide temperature ranges it is difficult to determine the exact rate of development.

Our previous paper on Guatemalan black flies described a very satisfactory method for rearing adults of *S. ochraceum* in the laboratory at 20 C and 25 C without excessive labor (Matsuo *et al.*, 1978). By using this method, *S. ochraceum* adults after feeding on volunteers with microfilariae of *O. volvulus* were kept in the laboratory and the parasites in the flies developed completely. This paper describes the development of parasites in the flies reared within the temperature range of 25±1 C and 20±1 C. These experiments were made in 1977, the first year of a five year project on onchocerciasis research and its control in Guatemala, Central America.

## Materials and Methods

Wild *S. ochraceum* adults feeding on exposed volunteers with microfilariae of *O. volvulus* were used. The captures were made at a coffee plantation "Santa Monica Iboné" in the municipality of Chicacao, an endemic area of the disease, in February and March of 1977. Engorged flies were collected one by one from the volunteers into maintaining tubes, described in previous paper (Matsuo *et al.*, 1978). These tubes with one adult

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Table 1 Development of larvae of *Onchocerca volvulus* in *Simulium ochraceum* maintained at 25±1 C

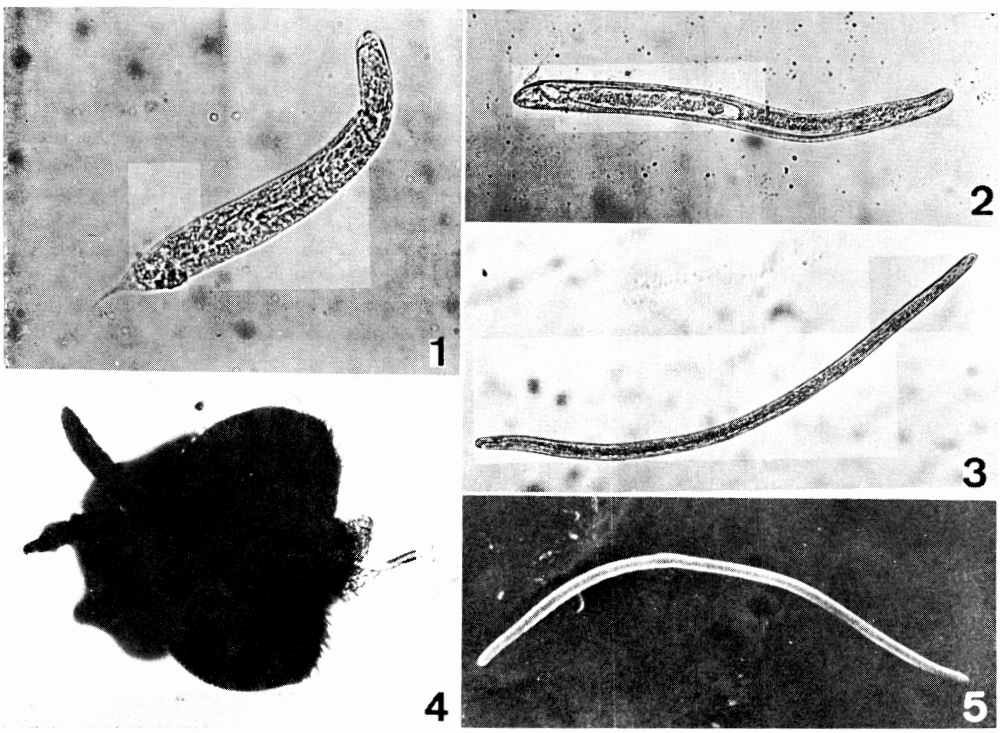
| Days after feeding on onchocerciasis volunteer | | 2 | 3 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. flies examined | | 4 | 10 | 10 | 10 | 13 | 7 | 10 | 11 | 17 | 13 | 20 | 8 |
| No. flies with 1st stage larvae | Abdomen | | 1 (2) | 2 (6) | | | | 1 (2) | | | | | |
| | Thorax | | 2 (16) | 3 (13) | 1 (2) | | | 1 (4) | | | | 1 (1) | |
| | Head | | | | | | | | | | | | |
| No. flies with 2nd stage larvae | Abdomen | | | | 1 (1) | | | | | | | | |
| | Thorax | | | 2 (11) | 3 (20) | 6 (17) | | | | | | | |
| | Head | | | | | | | | | | | | |
| No. flies with 3rd stage larvae | Abdomen | | | | | | | | 2 (2) | | 2 (2) | | 1 (2) |
| | Thorax | | | | | | | 1 (5) | | 1 (2) | 2 (3) | 2 (3) | |
| | Head | | | | | | 2 (3) | 6 (28) | 4 (12) | 2 (10) | 4 (7) | 1 (2) | 1 (1) |

Figures in parenthesis indicate the number of larvae found.

in each were put into cool boxes at 15–20 C for transportation from the plantation to the laboratory. In the laboratory, a cotton wad soaked once in 0.5% or saturated sugar solution then strongly squeezed was placed at the entrance of the tubes. These flies were kept in an incubator at 25±1 C and 20±1 C. The preserved living flies were dissected at about 24 hour intervals starting 1 day after ingestion of microfilariae. Flies were anesthetized in chloroform vapor, dissected in 0.9% saline, and the numbers and stages of larvae detected were recorded.

## Results and Discussion

At 25 C, a total of 133 living flies were dissected as shown in Table 1. Ninety-five 1st and 2nd stage larvae were found in 24 flies and 82 3rd stage larvae in 31 flies. Three days after ingestion, parasites were first seen as 1st stage larvae with a wide body and a definite tail in thoracic muscles of flies (Fig. 1). These larvae were 181–231 $\mu$m (207±19 $\mu$m) long, and similar to those at stages E-G depicted by Duke (1968). Five days later, 1st and 2nd stage larvae were seen in thorax, measuring 220–235 $\mu$m long and 308–400 $\mu$m long, respectively. Majority of 6 and 7 day old larvae were seen in thorax as 2nd stage larvae (Fig. 2). These larvae were 441–546 $\mu$m (499±36 $\mu$m) long, with esophagus and intestine, and were similar to the larvae at stages I-K depicted by Duke (1968). On the 8th day of infection, 3rd stage larvae were first seen in heads of flies. Some of the larvae moved into the saline during the dissection of flies, and showed active movement. On and after the following day, majority of the 3rd stage larvae were seen in head, but others in thorax and abdomen. These larvae measured




**Explanation of Figures**

Fig. 1 The 1st stage larva in thorax of fly on day 3. ×256.
Fig. 2 The 2nd stage larva in thorax of fly on day 5. ×140.
Figs. 3-5 The 3rd stage larvae in head of fly on day 8.
(3) : ×113.
(4) : Larva moving into saline from head. ×50.
(5) : Scanning electron micrograph. ×117.

558-683 μm (622±39 μm) long, and are shown in Figs. 3-5. The digestive tract of the larvae was patent, although it was out of the focus in Fig. 3. The lengths of the anterior esophagus, posterior esophagus and intestine comprised 18-22%, 41-44% and 28-33% of total body length, respectively. Measurements and morphological characters of these larvae were similar to those in African black fly, *S. damnosum* (Duke, 1967, 1968). Figure 5 is a micrograph taken by a scanning electron microscope. More detailed results by this tool will be described later.

Development of the larvae in flies was almost synchronous and proceeded in a regular process. On the other hand, 56 wild flies collected from the same plantation were dissected. No larvae were found among them, although the natural infection rate of flies with *O. volvulus* has not been studied on a large scale there. The results show that the infected flies studied in this experiment had been infected by the meal taken at the time of capture. Watanabe *et al.*, (1979) studied the development of ovaries of Guatemalan *S. ochraceum*, and estimated that the length of the gonotrophic cycle of the fly was 5 days at 22 C. Therefore, assuming that *S. ochraceum* adults deposit eggs and take subsequent blood meal on the same day, it is suggested that flies infected at their 1st blood meal are capable of transmitting the infective larvae when they take their 3rd or subsequent blood meals at about 25 C.

At 20 C, a total of 49 living flies were

dissected during 10–15 days after ingestion. Two 1st stage larvae were seen in thorax of 2 flies on day 12, 2 on day 15. Five 2nd stage larvae were seen in abdomen and thorax of 3 flies on day 15. One 3rd stage larva was seen in abdomen of 1 fly on day 11, and measured 555 $\mu$m long. Far smaller numbers of larvae were found in flies comparing with those in the flies kept at 25 C, and the development of these larvae proceeded more irregularly in the former. It was suggested that the minimum temperature for development of the *O. volvulus* larvae in flies might be below and close to 20 C.

## Summary

The adults of Guatemalan black flies, *S. ochraceum*, feeding to engorgement on infected volunteers were maintained at 25±1 C and 20±1 C. At 25 C, the 3rd stage larvae were first seen in heads of flies on day 8, and development of larvae was almost synchronous. At 20 C, only a small number of larvae found developed in an irregular fashion, and it is suggested that the minimum temperature for their development might be below and close to 20 C. Assuming that *S. ochraceum* adults deposit eggs and take subsequent blood meal on the same day, it is suggested that flies infected at their 1st blood meal are capable of transmitting infective larvae when they take their 3rd or subsequent blood meal at about 25 C.

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# *Onchocerca volvulus* の *Simulium ochraceum* 成虫への感染実験

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グアテマラにおける *Onchocerca volvulus* の主要媒介ブユ *Simulium ochraceum* 体内における発育を調べた．*O. v.* ミクロフイラリア陽性者を吸血した *S. o.* 野外成虫を 25 C, 20 C で飼育，剖見した結果，25 C では 8 日目に感染幼虫が，ブユ頭部から検出され，20 C では検出幼虫はごく少数であつた．本種ブユの gonotrophic cycle は 22 C で 5 日と推定されており，産卵後の吸血がその日に行われると仮定すると，ブユから人への感染は 25 C 前後ではミクロフイラリア摂取後，次々回の吸血時になると推定される．