

4. Review Articles Related to the Cooperation Project (Republications)

5) Review of the Biology and Ecology of Adult Blackflies in Relation to the Transmission of Onchocerciasis in Guatemala*

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Abstract: Recent studies on the biology and ecology of adult blackflies in relation to the transmission of human onchocerciasis in Guatemala are reviewed. First, earlier studies on the transmission of the disease since its discovery by Dr. R. Robles in 1915 are outlined. Second, eleven blackfly species caught on humans are evaluated for vector status on the basis of their natural and experimental infections with third-stage larvae of *Onchocerca volvulus*, and *Simulium ochraceum*,* *S. metallicum** and *S. callidum* are confirmed as natural vectors of the disease in Guatemala in descending order of importance, whereas *S. gonzalezi*, *S. haematopotum*, *S. veracruzianum* and *S. horacioi* are potential vectors. Third, the migration and fate of *O. volvulus* microfilariae ingested by female blackflies are highlighted on the basis of the findings of the cibarial armature of *S. ochraceum* and physiological incompatibility of *S. metallicum* as main barriers against microfilariae, both of which greatly decrease the number of ingested *O. volvulus* microfilariae developing to the third-stage larvae per female. Fourth, among many ecological factors of female blackfly populations, geographical and altitudinal distributions, habits of blood feeding, host preferences for blood feeding, preference for human body parts, parous rates, daily and seasonal fluctuations of biting activities, in particular, of parous females, gonotrophic cycle, longevity, flight range, and annual transmission potential are reviewed, and their influences on the transmission dynamics of the disease agents are considered. Fifth, effects of air temperatures on the *O. volvulus*–*S. ochraceum* complex are examined, with a special reference to the characteristic altitudinal distributions of the disease. The importance of reliable identification of both the vector blackfly species and filarial larvae found in female blackflies is emphasized to understand the transmission of the disease.

[*It is now known that these two species are actually species complexes.]

INTRODUCTION

Human onchocerciasis is caused by *Onchocerca volvulus* (Nematoda: Filarioidea) through bites of female blackflies (Diptera: Simuliidae), with primary symptoms of subcutaneous nodule formation, and dermal and ocular lesions including blindness in serious cases. Effective and safe drugs to cure this disease remain to be developed. The disease is distributed in Africa, the Arabic Peninsula, and Central and South America. Infected persons with this parasitic disease are estimated to be 15 to 20 million around the world. Its socio-economic effects are enormous in certain endemic areas.

In Africa, endemic areas are widely distributed in savanna and tropical forest zones south of the Sahara Desert, where investigations of the disease have been carried out for a long time. At present, the control of the disease through applications of insecticides against larvae of

Simulium damnosum, the major vector of the disease, in the endemic areas of the Volta River Basin, West Africa, has been in progress since 1973 by the Onchocerciasis Control Programme (OCP) supported by the World Health Organization, World Bank, etc.

In addition, endemic areas are scattered in six countries in the Western Hemisphere including Mexico, Guatemala, Venezuela, Brazil, Colombia and Ecuador. The symptoms and prevalence of the disease are somewhat different from one endemic area to another, and the transmission of the disease remains to be clarified in endemic areas in Ecuador and Amazonas State of Venezuela.

This article outlines the history of research on the transmission of human onchocerciasis carried out in Guatemala since 1915, when Dr. R. Robles discovered the disease for the first time in the Western Hemisphere, and reviews recent studies on the biology and ecology of adult blackflies in relation to the transmission of the disease in

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*Reproduced from Japanese Journal of Tropical Medicine and Hygiene Vol. 10, with kind permission from Japanese Society of Tropical Medicine. This is an English version translated by Hiroyuki Takaoka from his original review article written in Japanese, published in Jpn J Trop Med Hyg 1982; 10(1): 1–22.

Guatemala.

The readers may refer to the reviews of Dalmat [1] and Hamon [2] for the history of research on the transmission of human onchocerciasis in Guatemala, and to the reviews of Nelson [3], Tada [4] and Sasa [5] for general information on human onchocerciasis (history of research, causative parasite, pathology, transmission, clinical pictures, diagnoses, treatments, epidemiology and control).

HISTORY OF RESEARCH

Dr. R. Robles, a Guatemalan medical doctor, who discovered the disease in the Western Hemisphere, in 1915, suggested that blackflies are a probable vector of the disease based on his extensive epidemiologic surveys in the endemic areas [6, 7]. His insight was remarkable because vectors of human onchocerciasis in Africa was not known at that time, although vectors of two other filarial diseases, caused by *Wuchereria bancrofti* and *Loa loa*, were determined to be mosquitoes and deer flies, respectively [8, 9].

However, it was Blacklock that first proved that blackflies are the vectors of human onchocerciasis through experimental infections of *Simulium damnosum* with microfilariae of *O. volvulus* from patients in Sierra Leone, Africa [10]. He confirmed that the microfilariae of *O. volvulus* ingested by *S. damnosum* could develop to the infective larvae in the body of the blackflies.

In Guatemala, researchers from Harvard University investigated blackflies in relation to human onchocerciasis in 1931 and 1932, and reported the importance of the three blackfly species, *S. ochraceum*, *S. metallicum* and *S. callidum*, in the transmission of human onchocerciasis based on the discoveries of natural infections of these three species with *O. volvulus* larvae [11]. Subsequently, M. Giaquinto Mira, an Italian scientist, emphasized the relative importance of *S. ochraceum* by observing the strong anthropophily of this blackfly species in the endemic areas [12].

In the 1940s, blackflies and their role in the transmission of the disease have been studied by several investigators, such as R. De Leon, a Guatemalan entomologist, and H. Elishewitz from the Pan American Sanitary Bureau. Noteworthy are the extensive studies carried out by H. Elishewitz, who stayed in Guatemala in 1944 and 1945, including various objectives, i.e., 1) adult blackflies' preference for host animals in and outside the endemic areas, 2) seasonal and daily biting activities of anthropophilic blackfly species, 3) blood feeding habits (in relation to body parts, duration, climatic factors such as air temperature, humidity and illumination etc.), 4) geologic characteristics of the endemic areas, 5) duration required for development

of blackfly larvae, and 6) vector control. He concluded that *S. ochraceum* might be the main vector of human onchocerciasis based on its geographical distribution consistent with that of the disease, strong anthropophily, preference of any parts of the body for biting, and stable behavior while taking blood [13]. His successor, H. Dalmat, who had stayed in Guatemala from 1947 to 1953, had also extensively studied the taxonomy, ecology and transmission roles of blackflies supported by the NIH of the United States and Ministry of Health of Guatemalan government in the Memorial Institute of Robles' Disease, established at Yepocapa, Chimaltenango, a major endemic area of the disease in Guatemala. All of his works have been summarized in an excellent monograph titled "The black flies (Simuliidae: Diptera) of Guatemala and their role as vector of onchocerciasis" published by the Smithsonian Institution in 1955. This book has been long been the bible for those who would initiate studies of Guatemalan onchocerciasis. Dalmat established the taxonomy of blackflies in Guatemala, constructing keys to a total of 41 species including many new species discovered by him, and providing detailed descriptions and illustrations of diagnostic morphological characters of adults, pupae and larvae of all species. His new findings on geographical distributions, flight ranges, longevity, and resting places of blackflies have been highly valued, while his results on blood-feeding habits, aquatic habitat preferences and analysis of aquatic habitats in relation to environmental factors were similar to those already reported by Elishewitz [13]. Dalmat also justified the relative importance of *S. ochraceum*, *S. metallicum* and *S. callidum* based on the results of his ecological studies together with those of natural infections with *O. volvulus* larvae. At the same time, L. Gibson, who stayed in Guatemala from 1948 to 1952, first made an experiment to observe the development of *O. volvulus* microfilariae in the blackflies.

In 1966, De Leon and B. O. L. Duke carried out a cross infection experiment to distinguish the "Onchocerca-Simulium complex" of Guatemala from that of Africa. In first half of the 1970s, many foreign investigators, such as I. Tada (Japan), R. Garms (Germany), M. S. Omar (Germany), and O. Bain (France), who had experience with surveys of human onchocerciasis in Africa, visited Guatemalan endemic areas.

Research of human onchocerciasis in Guatemala met a new era by receiving two international project teams, i.e., "Onchocerciasis Research and Control Project" sponsored by the Japan International Cooperation Agency in collaboration with the Guatemalan government (the first term 1975–1980, headed by Dr. H. Takahashi, and the second term 1981–1983, headed by Dr. T. Suzuki), and that spon-

sored by the Center for Disease Control (CDC) of the United States (from 1975–, headed by Dr. R. Collins).

CONFIRMED AND POTENTIAL VECTOR BLACKFLY SPECIES OF *O. VOLVULUS*

To determine vectors of human onchocerciasis in Guatemala, many studies of natural and experimental infections have been carried out since studies by Strong *et al.* [11]. Three blackfly species, *S. ochraceum*, *S. metallicum* and *S. callidum*, have been implicated to be vectors of Guatemalan onchocerciasis mainly based on the results of natural infections. However, reevaluation is needed since no detailed information was shown concerning the identity and stages of larvae found in the body of blackflies in some reports as noted below.

Eleven of the 45 species of blackflies in Guatemala were known to be anthropophilic (Fig. 1) and eight of them were dissected in relation to natural infections of *O. volvulus* larvae (Table 1).

Natural infections

As shown in Table 1, three blackfly species, *S. ochraceum*, *S. metallicum* and *S. callidum*, were reported to harbor third-stage larvae of *O. volvulus* in wild-caught female blackflies.

It was Strong *et al.* that found adult blackflies naturally infected with *O. volvulus* larvae for the first time in Guatemala [11]. Third-stage larvae were detected in 89 of 1,658 female blackflies consisting of *S. ochraceum*, *S. metallicum* and *S. callidum*. However, whether one of the

three blackfly species or two or all were infected with third-stage larvae was not mentioned. In addition, at least some of the third-stage larvae appeared to be a different filarial species judging from the reported length range of the larvae (450–1,140 μ m) and the body part of the fly (Malpighian tubules) where third-stage larvae were found. These unknown filarial larvae probably are the same species reported by De Leon and Duke [14].

Third-stage *O. volvulus* larvae were most frequently found in *S. ochraceum*, with infection rates ranging from 0.02% to 3.2%.

Third-stage *O. volvulus* larvae were also found in *S. metallicum* by De Leon [15], Garms and Ochoa [16] and Tanaka *et al.* (unpublished data, cited by Ito *et al.* [17]) although first and second-stage larvae were more frequently reported from this blackfly species. It is doubtful that all the third-stage larvae found by De Leon [15] were those of *O. volvulus* because some larvae were recovered from Malpighian tubules. Rates of *S. metallicum* naturally infected with third-stage *O. volvulus* larvae were 0.07–0.34% (excluding rates reported by De Leon).

Natural infections of *S. callidum* with third-stage *O. volvulus* larvae were reported by De Leon [15], Collins [18] and Garms and Ochoa [16], with rates of 0.14–4.0%.

Among other species, *S. gonzalezi* and *S. haematopotum* were reported to be naturally infected with *O. volvulus* larvae by Gibson and Dalmat [19]. The stages of larvae recovered were not mentioned. *S. downsi* was reported to be naturally infected with first and second-stage *O. volvulus* larvae but not with third-stage larvae [20]. *Simulium horacioi*, described by Okazawa and Onishi [21],

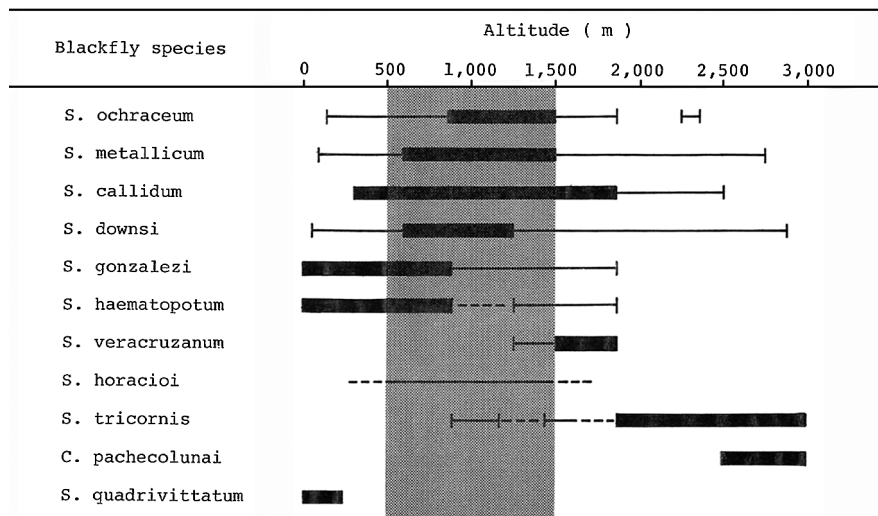


Fig. 1. Vertical distributions of eleven anthropophilic blackfly species in relation to the vertical range of the onchocerciasis endemic areas of Guatemala (Vertical range of endemic areas is shown by the shaded column; all data were based on Dalmat (1955) except for *S. horacioi* and *S. quadrivittatum*).

Table 1. Investigations on natural infection of Guatemalan blackflies with *O. volvulus* larvae

Author(s) and year	No. females dissected	No. females with <i>O. volvulus</i> larvae			% Females with larvae of any stages	% Females with third-stage larvae
		I	II	III		
<i>S. ochraceum</i>						
Strong <i>et al.</i> , 1934	1,658*	—	89	—	5.3	?
Gibson, 1951	1,839	—	7	—	0.38	?
Dalmat, 1955	6,815	—	30	—	0.44	?
De León, 1957	[26	0	0	1	3.8	3.8
	[70	0	0	1	1.4	1.4
	[61	0	0	1	1.6	1.6
Garms, 1975	[2,243	23	3	1	1.2	0.04
	[294	17	4	2	7.8	0.68
Collins, 1979	4,851	—	69	—	1.7	0.31
Garms & Ochoa, 1979	[1,445	80	11	13	7.2	0.90
	[154	13	3	5	13.6	3.2
Collins <i>et al.</i> , 1981	[1,282	—	9	—	0.7	0.39
	[3,569	—	60	—	1.68	0.28
Takaoka, 1982	1,551	9	0	1	0.6	0.06
<i>S. metallicum</i>						
Gibson, 1951	1,734	—	18	—	1.04	?
Dalmat, 1955	7,678	—	26	—	0.34	?
De León, 1957	[40	0	0	3	7.5	7.5
	[18	—	1	—	5.6	0.0
	[15	—	1	—	13.3	6.7
	[339	—	2	—	1.8	1.2
	[74	0	0	1	1.4	1.4
	[26	0	0	1	3.9	3.9
Garms, 1975	[242	1	0	0	0.4	0.0
	[679	3	0	0	0.4	0.0
	[269	1	0	0	0.37	0.0
	[1,826	2	0	0	0.11	0.0
Collins, 1979	1,855	—	23	—	1.2	0.0
Garms & Ochoa, 1979	[1,395	16	2	2	1.4	0.14
	[570	8	0	2	1.75	0.34
	[59	2	0	0	3.4	0.0
Tanaka <i>et al.</i> , unpub. cited by Ito <i>et al.</i> , 1980	2,638	—	10	—	0.45	0.07
Collins <i>et al.</i> , 1981	[795	—	5	—	0.63	0.0
	[1,060	—	26	—	2.45	0.0
Takaoka, 1982	1,663	—	2	—	0.12	0.0
<i>S. callidum</i>						
Gibson, 1951	161	—	1	—	0.62	?
De León, 1957	[1	—	1	—	100	0.0
	[?	0	0	1	?	?
Garms, 1975	27	1	0	0	3.7	0.0
Collins, 1979	702	—	3	—	0.57	0.14
Garms & Ochoa, 1979	100	—	2	—	6.0	4.0
Collins <i>et al.</i> , 1981	[251	—	1	—	0.4	0.0
	[451	—	2	—	0.44	0.0
<i>S. gonzalezi</i>						
Gibson & Dalmat, 1952	463	—	12	—	2.6	?
Garms, 1975	[13	1	0	0	7.9	0.0
	[134	2	0	0	1.5	0.0
Garms & Ochoa, 1979	206	8	0	0	3.9	0.0
<i>S. downsi</i>						
Collins, 1979	[503	—	1	—	0.2	0.0
	[491	—	1	—	0.2	0.0
<i>S. haematopotum</i>						
Gibson & Dalmat, 1952	1,276	—	2	—	0.2	?
<i>S. horacioi</i>						
Takaoka, 1982	743	0	0	0	0.0	0.0

* *Simulium metallicum* and *S. callidum* are also included.

was reported not to be naturally infected with *O. volvulus* larvae, based on 745 wild-caught females that were dissected [22].

It is a prerequisite to distinguish *O. volvulus* larvae from other *Onchocerca* species originating from horses and cattle when natural infections of blackflies with *O. volvulus* larvae are surveyed. Garms and Ochoa [16] compared natural infections of blackflies with *Onchocerca* larvae in and outside the endemic areas and reported that outside the endemic areas natural infections were not found in 304 females of *S. ochraceum* but were found in 0.35% (12/3,438) of *S. metallicum* and in 0.4% (1/242) of *S. callidum*. All first to third stages of *Onchocerca* larvae were indistinguishable from *O. volvulus* larvae. This result suggests that in endemic areas, all *Onchocerca* larvae recovered from blackflies, which are both anthropophilic and zoophilic, cannot always be identified as *O. volvulus*.

Experimental infections

Experimentally ingested *O. volvulus* microfilariae successfully developed to third-stage larvae in seven Guatemalan species of blackflies: *S. ochraceum*, *S. metallicum*, *S. callidum*, *S. gonzalezi*, *S. haematopotum*, *S. veracruzatum* and *S. horacioi* (Table 2). Strong *et al.* [11] tried to make experimental infections of blackflies but failed due to the difficulties in rearing adult blackflies. Gibson [23, 24] observed the larval development of experimentally ingested *O. volvulus* microfilariae to third-stage larvae in blackflies. In his experiments, 15,000 adult blackflies consisting of *S. ochraceum*, *S. metallicum* and *S. callidum* were allowed to feed on patients infected with *O. volvulus* and third-stage larvae were recovered from several adult blackflies that survived up to day 13 in air temperature of about 20°C. It was unclear which blackfly species supported the development to third-stage larvae. The great number of adult blackflies used for the experiments and the small number of blackflies that survived up to day 13 reflect the difficulties of the rearing of adult blackflies in

captivity. The result seems to be in contrast to that Blacklock obtained in Sierra Leone, in Africa in 1926. The difference in the results of experimental infections between Guatemala and Africa is probably attributable to the difference in the growth time required for *O. volvulus* microfilariae to reach third-stage larvae due to the difference in ambient air temperatures under which experimental infections were carried out. To obtain third-stage larvae, only one week was required for Blacklock to rear adult blackflies but almost two weeks for Gibson.

Gibson and Dalmat [19] also reported that three other blackfly species, *S. gonzalezi*, *S. haematopotum* and *S. veracruzatum*, successfully supported the development of experimentally ingested *O. volvulus* microfilariae to third-stage larvae. Ito *et al.* [17] showed successful larval development of experimentally ingested *O. volvulus* microfilariae in *S. horacioi*. In 1966, De Leon and Duke confirmed that larval development of ingested *O. volvulus* microfilariae to third-stage larvae occurred on day 7 or 8 after ingestion of microfilariae in *S. ochraceum*, *S. metallicum* and *S. callidum*. Similar results for experimental infections of *S. ochraceum* and/or *S. metallicum* were reported by other investigators [17, 18, 20, 25–28].

Overall, three blackfly species, *S. ochraceum*, *S. metallicum* and *S. callidum*, are regarded as natural vectors of *O. volvulus* and four other species, *S. gonzalezi*, *S. haematopotum*, *S. horacioi* and *S. veracruzatum*, are potential vectors.

MIGRATION AND FATE OF *O. VOLVULUS* MICROFILARIAE IN BLACKFLIES

The migration, developmental site, and fate of microfilariae in vectors differ among filarial species and anatomical and physiological conditions of vectors [29].

Migration route

Blacklock [10] clarified the migration route of inges-

Table 2. Result of natural and experimental infections of Guatemalan blackflies with *O. volvulus* larvae

Blackfly species	Natural infection		Experimental infection (Development to the third-stage larvae)
	Developing stages	Third stage	
<i>S. ochraceum</i>	+	+	+
<i>S. metallicum</i>	+	+	+
<i>S. callidum</i>	+	+	+
<i>S. gonzalezi</i>	+	?	+
<i>S. haematopotum</i>	+	?	+
<i>S. veracruzatum</i>	–	–	+
<i>S. horacioi</i>	–	–	+
<i>S. downsi</i>	+	–	?

ted *O. volvulus* microfilariae in the body of blackflies: i.e., after ingested by blackflies, *O. volvulus* microfilariae penetrate the gut wall, exit to the haemocoel and reach the thoracic muscles, where they undergo morphogenesis and become the third-stage larvae, which move to the head and penetrate the skin after passing through the proboscis.

In Guatemala, Strong *et al.* [11] confirmed the migration route when they dissected wild-caught blackflies. De Leon [15] found third-stage larvae in 23 out of 4,639 wild-caught female blackflies (*S. ochraceum*, *S. metallicum* and *S. callidum*). Among third-stage larvae, 74% were detected in the Malpighian tubules, 13% in the thorax and 13% in the abdomen. He inferred that *O. volvulus* microfilariae might have developed in the Malpighian tubules, or third-stage larvae might have moved to the Malpighian tubules after they developed in the thorax. He considered that third-stage larvae exit on to the human skin after passing through the wall of the abdomen. This hypothesis was ruled out by De Leon and Duke [14] who showed that the third-stage larvae found in the Malpighian tubules were not *O. volvulus*.

How the third-stage *O. volvulus* larvae exit from the head has been scarcely studied. Collins [20] sectioned the heads of blackflies and found the third-stage *O. volvulus* larvae in the labrum-epipharynx of the proboscis. He suggested that, contrary to other filariae transmitted by mosquitoes, third-stage *O. volvulus* larvae break the membrane which connects the labrum to the epipharynx on each side and exit on to the biting site of the human skin.

Intake of microfilariae

De Leon and Duke [14] reported that intake of *O. volvulus* microfilariae by *S. ochraceum*, *S. metallicum* and *S. callidum* varied from null to several hundred, although they fed on the same person infected with *O. volvulus*, and some females ingested a greater number of microfilariae than expected from the density of microfilariae in the skin of the infected person. The latter phenomenon, called "Over intake", was most frequently observed in *S. ochraceum* when feeding on a Guatemalan infected person, which was 20–25 times as many as the number of microfilariae ingested by *S. ochraceum* from an African infected person.

Strong *et al.* [11] found the same phenomenon for the first time and suspected the presence of substances, to which microfilariae are attracted, in the saliva of blackflies. They also noted the possibility of using this phenomenon as a xenodiagnosis of the disease. Later, the same phenomenon was confirmed by other investigators [25, 30, 31].

Fate of ingested microfilariae before and after reaching the developmental site of the blackfly

Not all the ingested microfilariae successfully develop to the third-stage. According to De Leon and Duke [14], the ratios of third-stage larvae among the microfilariae ingested were 0.65–2.1% in *S. ochraceum*, 1.2–2.5% in *S. metallicum* and 4.5% in *S. callidum*, and such reduced numbers of third-stage larvae were due to the decrease of microfilariae in the midgut of the blackflies. The similar low ratio, 0.2–2.4%, was also reported for *S. ochraceum* by Collins *et al.* [25]. In this connection, Bain *et al.* [32] observed that only 0.87–1.6% of the microfilariae ingested could pass through the wall of the midgut of the blackfly, and remaining microfilariae were digested in the midgut. Omar and Garms [33] were the first to indicate the difference of the fate of ingested microfilariae between *S. ochraceum* and *S. metallicum*, using a histopathological method: in *S. ochraceum*, the majority of microfilariae ingested were killed or injured by the toothed cibarium and only 2.6% could reach the thoracic muscles, whereas in *S. metallicum*, which lacks such anatomical armature on the cibarium and tends to ingest fewer microfilariae, compared to *S. ochraceum*, most microfilariae ingested could successfully pass the cibarium and reach the midgut and 74.5% of the ingested microfilariae could reach the thoracic muscles. Omar and Garms [33] also observed that the peritrophic matrix, formed inside the wall of the midgut soon after blood intake, might not play an important role as a barrier to prevent microfilariae from passing to the haemocoel, since most microfilariae could pass through the wall of the midgut to the haemocoel within a few minutes to six hours after ingested, when the matrix of the peritrophic matrix was still incompletely formed and soft.

De Leon and Duke [14] and Collins *et al.* [25] observed that most *O. volvulus* microfilariae that reached the thoracic muscles of *S. ochraceum* could develop to the third-stage larvae synchronously. Hashiguchi *et al.* [31] found that about half of *O. volvulus* microfilariae died after reaching the thoracic muscles of the same blackfly species.

Collins [20] compared the development of *O. volvulus* microfilariae in *S. ochraceum* and *S. metallicum*, which fed, each, on the upper and lower body parts of the same infected person. As a result, the numbers of larvae per fly after 24 hours and 8–10 days were 1.5 and 2.3 in *S. ochraceum* and 18.9 and 3.8 in *S. metallicum*. And, out of larvae found after 8–10 days, 90% and 26.3% were the third-stage larvae in *S. ochraceum* and *S. metallicum*, respectively. Over half of the remaining larvae in *S. metallicum* were still at the microfilarial and first stages, and 42% of microfilariae were distorted or degenerated. Overall, the number of the third-stage larvae in *S.*

metallicum was half the number of those in *S. ochraceum*, although the number of the microfilariae reaching the thoracic muscles in *S. metallicum* was 12 times as many as in *S. ochraceum*. Similar experiments carried out by Ito *et al.* [17] showed that 93.5% and 60% of the microfilariae reaching the thoracic muscles could develop to the third-stage larvae in *S. ochraceum* and *S. metallicum*, respectively. These findings strongly suggest that the physiological compatibility of *O. volvulus* microfilariae to the blackflies differs between the two blackfly species. De Leon and Duke [14] observed the synchronous development of *O. volvulus* microfilariae in *S. callidum*.

Effects of intake of microfilariae on longevity of blackflies

De Leon and Duke [14] elucidated the effects of the intake of microfilariae against the longevity of blackflies. The longevity of *S. ochraceum* was not affected when an average of 9 microfilariae per fly were ingested, but 14.2% and 39.3% of *S. ochraceum* died within 24 hours when an average of 170 and 390 microfilariae per fly, respectively, were ingested. On the other hand, 12% and 100% of *S. metallicum* died when 5 or 6 microfilariae per fly and 190 microfilariae per fly, respectively, were ingested. All *S. callidum* died when ingesting an average of 160 microfilariae per fly, as observed for *S. metallicum*. On day 2 and later, the longevity of the survived blackflies harboring larvae was almost the same as that of blackflies without larvae. Similar results were reported by Omar and Garms [33], Collins [20] and Ito *et al.* [17]. The maximum number of microfilariae ingested that could not affect the longevity of *S. metallicum* was calculated as 168 microfilariae per fly by Ito *et al.* [17].

Omar and Garms [34] histopathologically studied the effect of the high intake of microfilariae against the longevity of *S. metallicum* and *S. callidum*, and pointed out the following causes: 1) injury of the epithelium of the anterior part of the midgut by microfilariae that retreated from the main part of the midgut, 2) injury of the midgut epithelium accompanied by leakage of contents, 3) inhibition of the formation of the peritrophic matrix, 4) mechanical pressure of the hind gut by microfilariae and blood, 5) invasion and subsequent malfunction of various organs such as nerves, brain, eye, halteres, fat body, flight muscles etc.), any of which would singly or simultaneously become a fatal effect for blackflies. Hashiguchi *et al.* [31] reported that invasion of other organs or tissues such as the brain and legs by *O. volvulus* microfilariae was also observed in *S. ochraceum*, and suggested that this might be a cause of early death of this blackfly species.

BIOLOGICAL AND ECOLOGICAL FACTORS OF BLACKFLY POPULATIONS INFLUENCING THE TRANSMISSION OF ONCHOCERCIASIS

The role of vector blackfly species in the transmission of onchocerciasis differs depending on biological and ecological factors of blackflies such as geographical distribution, size of populations in endemic areas, seasonal and daily fluctuations of biting activity, blood-feeding habits, gonotrophic cycle, parous rates, longevity, flight range, etc. In Guatemala, these factors in relation to the transmission of onchocerciasis have been studied by Elishewitz [13] and Dalmat [1].

Geographical distribution

Human onchocerciasis in Guatemala is confined to 500–1,500 m in altitude on the southern slope of the Sierra Madre Mountain Range running north-west to south-east. The distribution of vector blackfly species is thought to be a major reason explaining the limited distribution of the disease, although no thorough studies were carried out to justify this characteristic altitudinal distribution of the disease.

The altitudinal distributions of 11 anthropophilic blackfly species in Guatemala are shown in Figure 1 based on the data provided by Dalmat [1]. As implicated by Elishewitz [13] and Dalmat [1], three species, *S. ochraceum*, *S. metallicum* and *S. callidum*, have their main breeding places between 500 and 1,500 m in altitude, consistent with the altitudinal range of the disease, although these three species also can breed at lower and higher altitudes. The distribution of *S. veracruzianum* partially overlaps the upper limit of the altitudinal distribution of the disease, whereas those of *S. gonzalezi* and *S. haematopotum* overlap the lower limit of the distribution of the disease. These three species were considered to be important in the transmission of the disease only in certain endemic areas where their population densities were high. Among other species, *S. horacioi* seems to have an altitudinal distribution similar to that of *S. ochraceum* because this species breeds in similar small streams, though extensive surveys of this species remain to be done. The four other species are ruled out as vectors of onchocerciasis in Guatemala since they breed outside the endemic areas in terms of altitude (lower than 500 m or higher than 1,500 m).

Blood feeding habits

It is during blood feeding by blackflies that *O. volvulus* microfilariae are ingested and third-stage of larvae escape. The transmission efficacies of vector blackflies

are greatly affected by their host preference, i.e., anthrophilic or zoophilic or both. Giaquinto Mira [12], Elishewitz [13] and Dalmat [1] studied the host preferences of Guatemalan blackflies and reported that *S. ochraceum* was a strongly anthrophilic species, whereas *S. metallicum* and *S. callidum* showed a strong preference for large animals such as horses and cattle, though all three species took blood from various animals.

The host preference of *S. horacioi*, another anthrophilic species, was not known, though one female of this species was captured from cattle [21]. However, *S. horacioi* certainly takes blood from other animals because unidentified filarial larvae, which were first reported from *S. metallicum* by Garms [35], were found in wild-caught females of *S. horacioi* [17, 22]. Takaoka [22] assessed the relative anthrophily of *S. horacioi*, as compared to *S. ochraceum* and *S. metallicum*, in Rio Verde, San Vicente Pacaya, where larval population of *S. metallicum* were most abundant, followed by *S. horacioi* (one-third that of *S. metallicum*) and *S. ochraceum* (one-twentieth that of *S. metallicum*), whereas the numbers of female blackflies collected using a human attractant were reversed in order of abundance, *S. ochraceum* being the most abundant, followed by *S. horacioi* (three-fourths that of *S. ochraceum*) and *S. metallicum* (one-half that of *S. horacioi*).

It was suggested that the anthrophily of *S. horacioi* was somewhat inferior to that of *S. ochraceum* but much superior to that of *S. metallicum*.

Species composition and relative abundance of blood feeding female blackflies

Dalmat [1] first reported the species of blood-feeding female blackflies and their relative abundance in endemic areas by analyzing 69,337 females captured in 3,249 collections: *S. metallicum* 65.3%, *S. ochraceum* 30.0%, *S. callidum* 4.0%, *S. downsi* 0.1%, *S. gonzalezi* 0.14% *S. haematopotum* 0.4% and *S. veracruzianum* 0.059%. From this result, *S. metallicum* and *S. ochraceum* were apparently suspected as the most important species in the transmission of human onchocerciasis. However, the species composition and their relative abundance vary by place and time. For example, Dalmat [1] noted that there were some endemic areas where *S. ochraceum* and *S. metallicum* were absent or in low densities, and any of the three other species, *S. gonzalezi*, *S. haematopotum* and *S. veracruzianum*, were the most abundant. Each endemic area should, therefore, be investigated to determine the vector blackfly species.

Daily fluctuation pattern of blood-feeding female blackflies

In patients infected with *O. volvulus*, daily periodic occurrence of microfilariae in the skin was not observed by Tada and Figueroa [36]. This is in contrast to the periodic types of lymphatic filariasis in which occurrence of microfilariae in peripheral blood vessels is periodic or subperiodic within 24 hours; thus, transmission efficacies of vector mosquito species are strongly affected by their daily blood-feeding periodicity. Female blackflies are theoretically able to ingest *O. volvulus* microfilariae from the skin of the patients anytime in a day (though, in reality, blackflies are known to be diurnal in biting activities); thus, daily blood-feeding activity patterns of blackflies, if any, do not appear to affect the transmission efficacies. However, it is important to know the daily blood-feeding pattern in relation to working time outdoors (which is usually in the morning) since no chance of *O. volvulus* microfilariae to be ingested exists if biting activities of blackflies are limited before or after working time during the daytime. It is also the case for the third-stage larvae in the blackflies to infect people.

Dalmat [1] observed that blood-feeding activities were highest between 8 and 10 hours in the morning in both *S. ochraceum* and *S. metallicum* but were bimodal, with the first peak in the early morning and the second peak in the evening in *S. callidum*. Tada *et al.* [37] reported a similar biting activity pattern in *S. ochraceum*. On the contrary, Collins *et al.* [38] noted that the highest peak of blood-feeding activity in *S. ochraceum* was between 7 and 9 hours in the morning but that in *S. metallicum* was between 2 and 5 hours in the afternoon. Collins *et al.* [38] also reported that the blood-feeding pattern of *S. ochraceum* infected with third-stage *O. volvulus* larvae was bimodal, with the first peak between 8 and 9 hours in the morning and the second peak between 0 and 1 hour in the afternoon. This report indicates that third-stage *O. volvulus* larvae can be transmitted to people not only when the blood-feeding activity of *S. ochraceum* was highest but also when it was lower.

Seasonal fluctuation pattern of blood-feeding activities of blackflies

The weather in Guatemala is divided into two seasons, rainy from May to October and dry from November to April. Under the circumstances, a question arises as to whether transmission of human onchocerciasis in Guatemalan endemic areas occurs throughout the year or during certain months. Seasonal abundance of the vector blackfly species was first investigated by Dalmat [1], who showed that *S. ochraceum* had two peaks in abundance, the

major peak in January and February in the dry season and the second one in August in the rainy season. He stressed the importance of the highest peaks of this blackfly species which generally agree with the harvest time of coffee fruit in the endemic areas, thus exposing laborers to be most frequently bitten by *S. ochraceum*. Similar seasonal patterns of abundance were shown for *S. callidum*. On the other hand, *S. metallicum* showed no such distinct seasonal pattern in abundance, i.e., relatively high from January to June (though a drop in May), then gradually decreasing in abundance toward December.

Takaoka [39] compared the seasonal abundance of *S. ochraceum* in three different places in San Vicent Pacaya endemic area, and concluded that the seasonal pattern of this blackfly species was different from one place to another. This suggests that the people working in coffee plantations are at risk to be bitten by *S. ochraceum* at harvest times in certain endemic areas but at other times of the year in other endemic areas.

The transmission time is not necessarily consistent with the peak in biting activity of the female blackflies: natural infection of *S. ochraceum* with third-stage *O. volvulus* larvae was highest in March, May and September, when biting rates were low, but was null in October and November, when biting rates were highest [38].

Body parts bitten by blackflies

The density of *O. volvulus* microfilariae in human skin was reported to be high on the upper body parts and low on the lower body parts [14, 40]. In this respect, the body parts preferred by blood-seeking blackflies were important in relation to the intake of *O. volvulus* microfilariae. Elishewitz [13], Dalmat [35], and De Leon and Duke [14] observed that the upper body parts were preferred by *S. ochraceum*, whereas the lower body parts were chosen by *S. metallicum* and *S. callidum*. On the contrary, Tada *et al.* [36] observed *S. ochraceum* biting any exposed body parts irrespective of the height above the ground. Elishewitz [13] and Dalmat [1] regarded *S. metallicum* and *S. callidum* as inefficient vectors due to their preference for the lower body parts where densities of *O. volvulus* microfilariae were low. As already noted, *S. metallicum* tended to die when it ingested many *O. volvulus* microfilariae and its preference for the lower body parts with low densities of *O. volvulus* microfilariae was likely to be beneficial to the longevity of this blackfly species.

It is interesting that *S. ochraceum* preferred the upper body parts, where densities of *O. volvulus* microfilariae were usually high, but this blackfly species has not been affected by an over intake of microfilariae because many

microfilariae are injured or killed by sharp tubercles on the cibarium.

Blood-feeding behavior and engorgement time

The blood-feeding behavior, in particular, engorgement time, of vector blackflies affects not only intake of *O. volvulus* microfilariae but also migration of third-stage larvae. Elishewitz [13], Dalmat [1] and De Leon and Duke [14] reported that the blood feeding was stable for *S. ochraceum* but unstable with incomplete feedings often seen for *S. metallicum* and *S. callidum*. According to Dalmat [35], the average time required was 4.8 minutes (range, 1–19 minutes) for *S. ochraceum*, 4.3 minutes (range, 1–31 minutes) for *S. metallicum*, and 4.5 minutes (range, 1–15 minutes) for *S. callidum*. Tanaka *et al.* [30] reported that about 70% of *S. ochraceum* took 3 to 4 minutes to fully blood feed on persons infected with *O. volvulus*.

Parous rates

Parous and nulliparous females are those that experienced one or more blood-feedings and subsequent ovipositions, or not, respectively. The rate of parous female blackflies is epidemiologically important since *O. volvulus* larvae are detected only among parous females. Garms [35] first distinguished parous females from nulliparous ones of four blackfly species caught on human attractants by examining the presence or absence of follicular dilatations of the ovaries. In four endemic areas of Chimaltenango and in one endemic area of Quezaltenango, parous rates were 31–59% for *S. ochraceum*, 18–38% for *S. metallicum*, 21–61% for *S. callidum* 28–67% for *S. gonzalezi*. Garms and Ochoa [16] also examined the follicular dilatations of the same four species in six provinces including Chimaltenango and reported the parous rates: 46–79% for *S. ochraceum*, 23–73% for *S. metallicum*, 25–60% for *S. callidum* and 40–75% for *S. gonzalezi*. Collins *et al.* [38] compared the parous rates of three blackfly species between Los Trrales and El Vesuvio and obtained the following results: 33.8% and 37.0% for *S. ochraceum*, 37.0% and 14.3% for *S. metallicum* and 39.8% and 14.6% for *S. callidum*. They suggested that the parous rates differed by localities and times of collection.

Garms and Ochoa [16] observed no distinct daily pattern in the parous rates of *S. ochraceum*, though slightly higher early in the morning, whereas they observed a distinct bimodal daily pattern of the parous rates of *S. metallicum* with the first peak in the morning and the second late in the afternoon. On the contrary, Collins *et al.* [38] found on the basis of yearly collections that rates of parous females of *S. ochraceum* were low, being 27% or

below during 6–11 hours in the morning, but suddenly began to increase from 11 hour and reached a peak of 64% in 13–14 hours, whereas those of *S. metallicum*, *S. callidum* and *S. downsi* did not show such a distinct pattern. All these data suggest that natural infection rates of blackfly species with *O. volvulus* larvae are likely to be greatly affected by the timing of collections of female blackflies within a day if the blackfly species shows a distinct daily fluctuation pattern in the parous rates.

Gonotrophic cycle

The duration of time required between one blood feeding and another (gonotrophic cycle) is important in relation to the timing of the third-stage *O. volvulus* larvae becoming infective. The time of the gonotrophic cycle (usually expressed as days or hours) is inferred by observing the duration required for ovarian development after female blackflies take a blood meal. Cupp and Collins [41] observed the state of follicles in the ovaries immediately after emergence, the development of follicles to mature eggs after blood engorgement, and changes in follicles after mature eggs were laid. The development of mature eggs took 48 hours under an air temperature of 25°C [41] and four days under an air temperature of 22°C [42]. Monroy [27] and Takaoka *et al.* [28] reported that the ovarian development was 2 days under an air temperature of 22°C or above, and was longer from 3 days to 9 days as the air temperature decreased from 22°C. Garms and Ochoa [16] reported that *S. ochraceum* and *S. metallicum* laid eggs on days 2, 3 and 4 after blood engorgement in air temperature of 20–27°C. Garms [35] observed that follicular dilatations of females of *S. ochraceum* caught in the morning were small but those taken in the afternoon were large, and inferred that females with small and large dilatations laid eggs one day before and in the morning of the same day, respectively. Garms [35] thought that the oviposition of *S. ochraceum* occurs early in the afternoon (12–14 hours), based on the high rate of females with large follicular dilatations in the afternoon, as reported by Dalmat [1].

The uptake of carbohydrates of blackflies during the gonotrophic cycle is scarcely known. Cupp and Collins [41] observed, using the Anthron test, that 70% of nulliparous and 65% of parous females of *S. ochraceum* took fructose, and suggested that even after the first gonotrophic cycle, nectar will be needed as an energy source to be used for the subsequent flight while seeking a source of blood.

The resting places of female blackflies between blood engorgement and egg maturation are not well documented except the observation by Dalmat [1], who recorded female blackflies resting on the ground during the night and

on the leaves and twigs of trees during the day time, i.e., 37 *S. ochraceum* at 4–34 m in height, and 11 *S. metallicum* at 6–16 m in height. No information was available as to whether these blackflies were nulliparous or parous, or blood engorged or not, or gravid (containing mature eggs) or not.

Longevity of blackflies

The longevity of female blackflies is one of the important factors in relation to the transmission efficacies of vector species, e.g., the rates of females surviving until and after the third-stage *O. volvulus* larvae developed. However, it is nearly impossible to obtain the actual daily longevity rate of female blackflies carrying the third-stage *O. volvulus* larvae due to the difficulties in inducing newly emerged females to blood feed on a patient infected with *O. volvulus* in the laboratory, even though rearing of female blackflies was improved by Figueroa *et al.* [43] and Matsuo *et al.* [44].

Dalmat [1, 45] and Gibson and Dalmat [19] estimated, using a mark-release-recapture method, the maximum longevities of three vector species: 62 days for *S. ochraceum*, 85 days for *S. metallicum* and 63 days for *S. callidum*. These data suggest that female blackflies survived in the field much longer than expected. Gibson and Dalmat [19] also marked female blackflies that blood fed on a patient infected with *O. volvulus* and found that all females captured on day 3 or later were negative for *O. volvulus* larvae. They considered female blackflies that ingested *O. volvulus* microfilariae to have shorter longevity relative to those that did not ingest *O. volvulus* microfilariae.

Watanabe *et al.* [42] estimated the daily longevity to be 0.866 for *S. ochraceum*, based on the duration (five days) of the gonotrophic cycle in air temperature of 20°C and the parous rate (48.7%). It should be remembered that this method was used under the assumption that the daily longevity rate is constant. Further, caution is needed to use this method because the duration of the gonotrophic cycle varies under different air temperatures, and the parous rate also fluctuates by locality, season, and the time of the day.

Flight range

Flight range of vector blackflies is one of the important factors influencing transmission efficacies and the range of endemic areas. Dalmat [45, 46] and Gibson and Dalmat [19] recorded the maximum flight range of three vector species by using a mark-release-recapture method: 10.1 km for *S. ochraceum*, 11.7 km for *S. metallicum* and 11.9 km for *S. callidum*. Among those released after blood feeding on a patient infected with *O. volvulus*, three fe-

males (two *S. ochraceum* and one *S. callidum*) carrying *O. volvulus* larvae were recaptured at 4.64 km on day 3 and at 4.32 km on day 4. They concluded that the flight range of females infected with *O. volvulus* larvae is limited, compared with that of females uninfected.

Annual transmission potential

Annual infection biting density (AIBD) and annual transmission potential (ATP) are each defined as the number of female blackflies carrying third-stage *O. volvulus* larvae biting a person per year, and the number of third-stage *O. volvulus* larvae infecting a person per year. Both numbers are used as important indices to assess the transmission dynamics, in particular the intensity of infection, in the endemic areas [47]. In Africa, AIBD and ATP were calculated in each endemic area and their strong relationships with the inhabitants' rates with *O. volvulus* microfilariae, dermal symptoms, nodule formation and blindness, all due to *O. volvulus* infection, were shown [48, 49].

In Guatemala, Collins [18] calculated the AIBD and ATP to be 540 and 1,188 in Finca Los Tarrales during a one-year investigation. However, these figures are not directly comparable with those reported from Africa since the calculation method used by Collins [18] was different from that used in Africa where both AIBD and ATP were expressed as the sum of the figures obtained each month of the year. Natural infections of *S. ochraceum* with third-stage *O. volvulus* larvae in Guatemala are very low (0.02–3.2%). This may require collecting and dissecting a considerable number of female blackflies per month to attain the actual AIBD and ATP in endemic areas in Guatemala.

FACTORS INFLUENCING THE *O. VOLVULUS*— *S. OCHRACEUM* COMPLEX

The transmission of onchocerciasis is affected by human and natural environmental factors. However, only a few studies have been done on these factors.

Densities of *O. volvulus* microfilariae in the skin of patients

The densities of *O. volvulus* microfilariae in the skin of patients are considered to be important in influencing the intake of microfilariae by female blackflies and subsequent larval development to third-stage larvae. Collins *et al.* [25] studied the relationships of the densities of microfilariae to the number of ingested microfilariae, the number of microfilariae migrating to the thorax and the number of third-stage larvae developing, by allowing wild-caught *S. ochraceum* to blood feed on 10 patients with different microfilarial densities. As a result, no correlation

was found between densities of microfilariae and the number of microfilariae ingested, but a clear correlation was found between the number of microfilariae ingested and microfilariae migrating to the thorax and also the number of the third-stage larvae. It is noticeable that even the patients with low densities of microfilariae, such as 0 and 0.5 microfilariae per mg of skin, yielded 1.7 and 44.7 ingested microfilariae per female, and an average of 0.04 and 0.17 third-stage larvae per female, respectively, and allowed 5.3% and 9.2% of *S. ochraceum* to carry third-stage larvae, respectively. Further studies might be required considering the uneven distribution of *O. volvulus* microfilariae in the body parts, the exposed body parts and the female blackflies' preferences for the body parts.

Air temperature

Among natural factors, only air temperature has been studied for its influence on a Guatemalan *O. volvulus*–*S. ochraceum* complex [27, 28]. Monroy [27] reported that the growth rate of *O. volvulus* microfilariae to third-stage larvae in *S. ochraceum* increased as the air temperature increased from 18°C to 30°C in the laboratory, and noted that an air temperature of 22–24°C would be suitable for transmission, based on the high longevity of *S. ochraceum* under this range of air temperatures.

Takaoka *et al.* [28] made a similar experiment and found successful larval development between 20°C and 28°C, and expressed the relation of the larval growth rate with air temperature as $y = -0.3760 + 0.0222x$ (y : growth rate, x : air temperature), and calculated the critical developmental point for *O. volvulus* in *S. ochraceum* as 17°C, below which no larval development occurred. Takaoka *et al.* [28] attempted to measure the effect of air temperature on transmission as infective fly day period calculated by multiplying the rate of female blackflies surviving until the microfilariae developed to the third-stage larvae, the rate of female blackflies with third-stage larvae among living female blackflies, and the life expectancy of the female blackflies with third-stage larvae after the day when the first third-stage larva was detected. As shown in Table 3, air temperatures of 22°C and 25°C were evaluated to be most suitable for transmission. Takaoka *et al.* [28] also measured the infective fly day period under different air temperature conditions by simulating the natural daily fluctuation of air temperatures in endemic areas in Guatemala, i.e. setting a constant air temperature of 25°C during the daytime and five different air temperatures of 10°C, 12°C, 14°C, 16°C and 18°C during the night time. As a result, exposure of female blackflies infected with *O. volvulus* microfilariae to the low night air temperatures increased both the longevity and life expectancy of female

Table 3. Comparison of infective fly day period in nine groups of *S. ochraceum* which were fed on a carrier of *O. volvulus* microfilariae and were kept at various constant temperatures or at fluctuating temperatures by day and night (arranged from Takaoka *et al.*, 1982)

Temperature (°C)	Duration of larval development (in day) (n)	Survival probability on day n (1)	% Females with infective larva(e) among survivors through n days (2)	Life expectancy with infective larva(e) (in day) (3)	Infective fly day period [10 × (1) × (2) × (3)]
20	16 (13)*	0.229 (0.295)	33.3 (26.3)	4.1 (7.1)	31.3 (55.1)
22	8	0.508	43.3	4.5	99.0
25	6	0.501	30.0	4.4	66.1
28	4	0.051	33.3	0.5	0.9
10-25	13	0.249	11.8	3.5	10.3
12-25	17 (13)	0.265 (0.315)	43.8 (38.8)	5.2 (9.2)	60.4 (112.4)
14-25	13	0.505	45.7	5.4	174.6
16-25	13	0.656	47.7	5.8	181.5
18-25	10	0.438	38.1	3.4	56.7

* Figure in parentheses shows the value calculated if the duration (n) were 13.

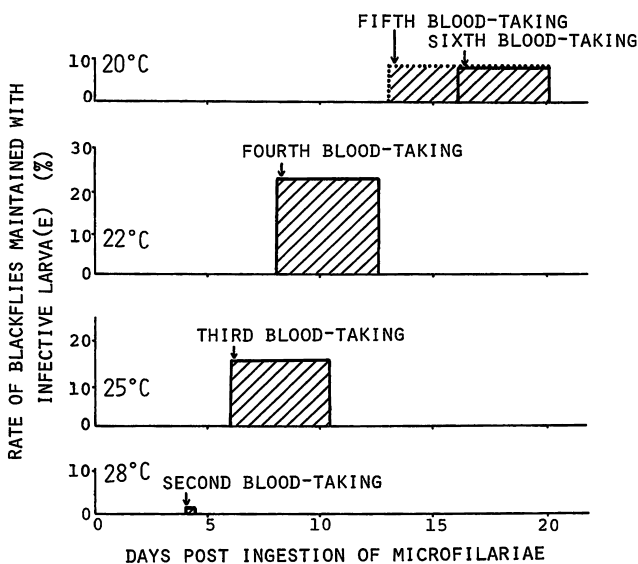


Fig. 2. Infective fly day periods in four groups of *S. ochraceum*, which were fed on a carrier of *O. volvulus* microfilariae and were kept at various constant air temperatures, and their relation to the number of gonotrophic cycles.

blackflies, although it caused the delay of larval development in female blackflies. Larval development was completed on day 13 even under night air temperatures of 12°C, 14°C and 16°C which were lower than the critical developmental point. Further, much higher values of infective fly day period were obtained under the day-night fluctuating air temperatures of 25°C/14°C, 25°C/16°C and 25°C/18°C than those observed under constant air temperature of 25°C (Table 3). In Figs. 2 and 3, infective fly day periods of different air temperature conditions are shown

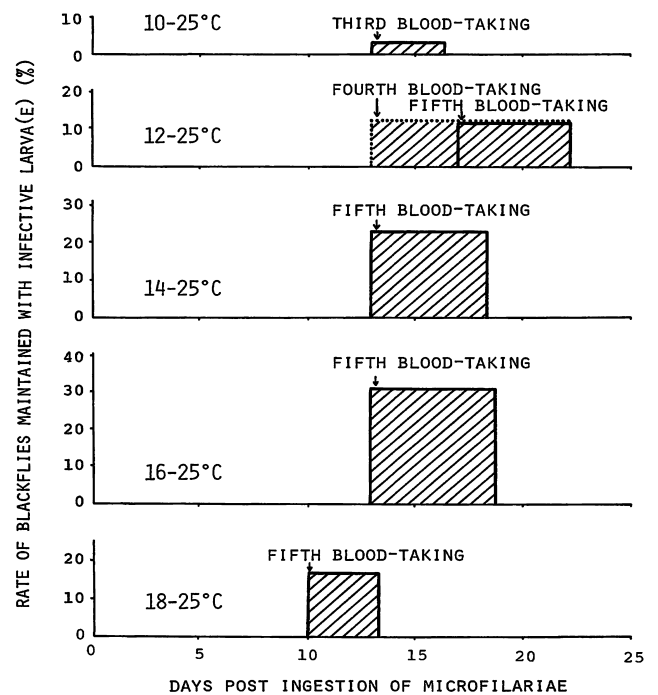


Fig. 3. Infective fly day period in five groups of *S. ochraceum*, which were fed on a carrier of *O. volvulus* microfilariae and were kept at different air temperatures of night time (14 hours) but under a constant air temperature of 25 °C during the day time (10 hours), and their relation to the number of gonotrophic cycles.

together with the estimated number of gonotrophic cycles in terms of the number of blood-feedings. Takaoka *et al.* [28] discussed the reason why the altitudinal distribution of endemic areas is confined to the range between 500 m

and 1,500 m, where night air temperatures decreased below the critical developmental point of 17°C.

It was concluded that the intolerance to high air temperatures by the vector *S. ochraceum* and no (or delayed) larval development of *O. volvulus* under low air temperatures prevent expansion of endemic areas to lower and higher areas, respectively.

Takaoka *et al.* [50] calculated the accumulated effective heat time for development of *O. volvulus* in *S. ochraceum* based on air temperature data recorded outdoors at five different altitudes ranging from 350 m to 1,500 m and reported 4.4–28 days as the estimated duration of *O. volvulus* larval development at these altitudes. Further field experiments are needed to justify this estimation by directly observing the larval development of *O. volvulus* in *S. ochraceum* at different altitudes of the endemic areas.

CLOSING REMARKS

In reviewing earlier studies on the transmission of human onchocerciasis in Guatemala, Dalmat's outstanding contribution is reconfirmed, and Elishewitz' works, though unpublished, and thus almost unknown, are also highly valued.

The improved methods of rearing adult blackflies in captivity for a few weeks made experimental infection studies possible, thus greatly advancing knowledge of the transmission of human onchocerciasis in Guatemala, such as evidence of various anthropophilic blackfly species' capabilities to support the larval development of *O. volvulus* to third-stage larvae, and effects of air temperature on the growth of *O. volvulus* microfilariae to third-stage larvae (including an estimation of the critical developmental point of 17°C) and the vector's longevities. And, an approach using histopathological methods yielded a striking discovery that the fate of *O. volvulus* microfilariae ingested by female blackflies greatly differs depending on the vector blackfly species, i.e. the cause of the decrease in the number of ingested microfilariae is due to the presence of the cibarial armature in *S. ochraceum* and the physiological incompatibility in *S. metallicum* [20, 33]. Similar studies will clarify the fate of *O. volvulus* microfilariae in other vector blackfly species in relation to the transmission efficacy of each vector species in the near future. Distinction of parous female blackflies from nulliparous ones is also highly valuable for clarifying the time of the day of infections with third-stage *O. volvulus* larvae, which is not necessarily consistent with the peak hours of biting activities [38]. A few long-term investigations to determine AIBD and ATP have been done but, unfortunately, the methods used

were not the same as those used in Africa.

One of the problems left for future studies is to reliably identify the filarial larvae found in wild-caught zoophilic blackfly species such as *S. metallicum* and *S. callidum*. To determine to what extent these two species are implicated in the transmission of human onchocerciasis in Guatemala, it is essential to distinguish larvae of *O. volvulus* from those of *O. cervicalis*, *O. gutturosa* and other related species. It is also important to reliably identify the blackfly species implicated in the transmission. For example, the females of *S. metallicum* are morphologically similar to those of related species, i.e., *S. horacioi*, *S. jobbinsi* and *S. puigi*. Moreover, most vector species such as *S. ochraceum*, *S. metallicum*, *S. callidum* and *S. exiguum*, which are widely distributed from Central to South America, are likely to represent species complexes consisting of more than two sibling species,* as in the case of *S. damnosum* in Africa, shown mainly by cytotaxonomic studies. Each sibling species of the vector blackfly species, if verified, will need to be studied as to its blood-feeding preference for humans, compatibility for infection with *O. volvulus*, daily biting activity, AIBD and ATP.

Future studies of human and natural environmental factors in the complicated ecosystem of the endemic areas may also contribute to an understanding and control of human onchocerciasis in Guatemala.

[*It is now known that they do consist of sibling species.]

ACKNOWLEDGMENTS

The content of this review article was presented as an invited special lecture at the 31st Annual Southern Japan Regional Meeting of Japan Society of Medical Entomology and Zoology, held in Kumamoto, Japan, on November 7, 1981. I am grateful to Prof. Isao Tada, Kumamoto University and the president of this meeting, for his invitation. My appreciation goes to Ms. Chiharu Aoki, Oita Medical College, for her assistance.

[Thanks are due to Prof. Peter H. Adler, Clemson University, Clemson, SC, USA, for reading the English version of the manuscript and providing valuable comments].

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