Comparative studies on the affinities of two black flies, *Simulium metallicum* and *S. ochraceum* for the larvae of *Onchocerca volvulus* in Guatemala

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Abstract: The affinity of *Simulium metallicum* for *Onchocerca volvulus* was compared with that of a good intermediate host, *S. ochraceum*, by maintaining infected flies under 25±0.5°C. The larval development in *S. ochraceum* was synchronous, but that in *S. metallicum* was asynchronous and malformed, stunted larvae often appeared. The results suggested that the ability for *S. metallicum* to transmit *O. volvulus* would come to about 1/3.5-1/5 of that of *S. ochraceum*. Some information about the affinity of *S. horacioum* Okazawa and Onishi, 1980 for *O. volvulus* that closely resembles *S. metallicum* was given.

For determination of the vectorial capacity, it is basically important to clarify the affinity of vector for parasite. *Simulium ochraceum* and *S. metallicum* are two of the most abundant anthropophilic species in endemic areas of human onchocerciasis in Guatemala. The former has been considered the more important vector of the disease, followed by the latter (Dalmat, 1955; De Leon and Duke, 1966; Garms, 1975). Then, it was demonstrated experimentally that *S. ochraceum* was the compatible host capable of supporting development of *O. volvulus* Guatemalan strain (De Leon and Duke, 1966; Collins et al., 1977; Matsuo et al., 1980). It was also reported that *S. metallicum* was a very poor experimental host for *O. volvulus*, because the development of parasite larvae was arrested and only a few reached the infective stage in *S. metallicum*, and also the damage of flies themselves by the intake of many microfilariae was often observed (De Leon and Duke, 1966; Omar and Garms, 1977). However, there were only a few data about the developmental potential for the parasite in *S. metallicum*.

The Onchocerciasis Control Project in Guatemala confronted the problem whether *S. metallicum* should be regarded as one of control targets or not. Therefore, it was planned to determine experimentally the affinity of *S. metallicum* for the larvae of *O. volvulus*. Although such preliminary experiments as mass rearing of pupae of black flies to adults and feeding them on human body in the laboratory were successful (unpublished), field-collected flies were used in the experiment, because of the difficulty in collecting large numbers of pupae within a limited term.
In the present paper, the results of laboratory experiments made on the affinity of *S. metallicum* for the larvae of *O. volvulus* are reported comparing with that of a good intermediate host, *S. ochraceum*, for the parasite.

**MATERIALS AND METHODS**

Wild flies of *S. metallicum* and *S. ochraceum* were caught after feeding on four volunteers with microfilariae of *O. volvulus*, at five coffee plantations, Fincas Peña Blanca, Injerto, Jazmines, Berlin and Hamburg in the municipality of San Vicente Pacaya, the pilot area for the project, ten times during the period from 28 March to 1 June 1978.

During the period from 15 June to 7 July, each microfilarial density in the four volunteers was examined at the definite part on every side of their bodies (shoulder, buttock, arm and calf) by skin-snipping. Geometrical mean numbers of microfilariae per 1 mm² of two pieces of skin at shoulder, buttock, arm and calf were 21.5, 43.6, 0 and 0.3 in Volunteer I, 11.5, 78.7, 2.4 and 0.9 in II, 18.8, 12.9, 6.3 and 0.1 in III, and 6.9, 21.7, 8.0 and 0.7 in IV, respectively.

Engorged flies were caught one by one with each maintaining tube (described below) from every bitten part of body (upper torso, arm and lower leg). These tubes were stored in a dark box kept at 15-20°C and carried to the laboratory within several hours. Then, after these flies were quickly confirmed to be alive, they were maintained in an incubator of 25±0.5°C and darkness, except for some flies that were dissected for the number of the microfilariae.

In this study, adult flies were maintained by the method devised by Matsuo et al. (1978) of this project, with slight modification as follows. The container for maintenance was a plastic polystyren tube (43 mm long, 15 and 13 mm diameter at its top and bottom) with a strip of filter paper (28×34 mm) laid inside, and the entrance of the tube was covered with a piece of nylon tricot, fixed by a rubber band and then a polyvinyl chloride cap with a hole (about 3 mm diameter) for aeration in the center. As the food of flies, a cotton wad strongly squeezed after drenched in 5% glucose solution was placed between the nylon tricot and the cap. The maintenance tubes each including one fly were placed in an adequate plastic tray, which was wrapped in a towel previously soaked with water and hard squeezed, then put into plastic bag. The cotton wad and the towel were renewed every 4 days.

Each day, the number of live flies was recorded, and dead flies were preserved in a freezer. Excepting for every about 5 flies killed at about 24 hr intervals for the first 7 days starting from 1 day after engorgement, all survivals were killed at 8-10th day. These flies killed were also preserved in the freezer. The freezing flies were dissected in 0.9% saline, in which the numbers and stages of larvae of *O. volvulus* were verified, and their length and width were measured. The larval stages were decided on referring to Nelson and Pester (1962), and Duke (1967, 1968).

All counts of microfilariae and larvae were transformed by log (n+1) for the frequency to approach the normal distribution, and geometric mean was calculated, after De Leon and Duke (1966).

**RESULTS**

*Survival of S. metallicum and S. ochraceum after ingesting microfilariae of O. volvulus*

Figure 1 shows the percent daily survival of *S. metallicum* and *S. ochraceum* which were fed on various body parts of microfilaria-carriers, and maintained for 8-10 days in the incubator described above. In the groups of *S. metallicum* which fed on arms or lower legs with low microfilarial skin density and subsequently ingested no or a very small number of microfilariae (0 or 2.3 geometric mean (GM) mf. per fly), none of flies died off during the first 24 hr. However, in the groups of more microfilarial intakes (over 10.8 GM mf. per fly) from upper torsos with high microfilarial skin density, greater numbers of microfilariae were ingested, and higher mortality of flies at 24 hr after feeding occurred. In the case of *S. ochraceum*, there was no difference in the mortality at 24 hr after feeding among the groups of different microfilarial intakes, as the all groups
The survivals of *Simulium metallicum* and *S. ochraceum* after ingestion of microfilariae of *Onchocerca volvulus*

GM: geometric mean, Mf.: microfilariae, T: upper torso, A: arms, L: lower legs
* Examined were only flies that died off within several hours after infected blood meal.

(with a range of 8.3–390.9 GM mf. per fly) showed high survival rates, over 96%.

In *S. ochraceum* dissected soon after feeding, it was observed that many of microfilariae ingested in the stomach were injured presumably by cibarial armatures as pointed by Omar and Garms (1975). In contrast, almost all microfilariae ingested by *S. metallicum* lacking such armatures were unhurt. Therefore, all of *S. metallicum* heavily infected with microfilariae died within several hours after feeding, and many microfilariae were found on dissection to have invaded the various organs, such as haemocoelce (abdomen, thorax, head), malpighian tubules, ovaries, halters, legs and compound eyes (Fig. 2 A–D). GM (minimum–maximum) of microfilarial intakes in 48 females of *S. metallicum* dying off within the first 24 hr was 364.2 (44–1,948). Then, it was tried to speculate the maximum number of microfilarial intake for the survival more than 24 hr, from the frequency of microfilarial intakes in 72 flies which were killed at 5–10 hr after feeding (Fig. 3). The mortality at 24 hr after feeding in 237 flies concurrently collected with above 72 flies was 12.1%. Being based on this, 9 out of 72 flies (72×0.121=8.7) would have died within 24 hr after feeding owing to many microfilariae, even if they were not killed at 5 to 10 hr after. Those 9 flies were shown in Fig. 3 with shaded pillars. The maximum number of microfilarial intakes in flies capable of surviving up to 24 hr after feeding was speculated as 168.

**Development of O. volvulus in the both fly species**

In order to observe the development of *O. volvulus*, flies of both species that died during the maintenance or were killed on various days after feeding were dissected. Third-stage larvae in *S. ochraceum* were first seen in thoracic muscles of flies on day 7, and in heads on day 8. The periods of development in *S. metallicum* were similar to those of *S. ochraceum*. In *S. ochraceum*, and less frequently in *S. metallicum*, infective larvae were observed to come out from mouth parts of flies when their heads were lightly pushed, as if the larvae had waited for a chance to get out of the proboscises (Fig. 2 E). It is reasonably certain from this evidence that *S. metallicum* also had the ability to transmit *O. volvulus*. Accordingly, both *S. ochraceum* and *S. metallicum* are capable of transmit-
All photographs were from *S. metallicum*.

A and B: The middle(A) and the tip(B) of malpighian tubule fully packed with microfilariae in a fly died within several hours after infection. C: A halter invaded by some microfilariae. D: Microfilariae invading into the femur of mid leg. E: An infective larva coming out from mouth part of fly. F-a: Malformed and dead first-stage larvae detected from a fly on the 10th day after infection. F-b: A malformed second-stage larva from a dying fly on the 7th day after infection.
Fig. 3 Histogram showing the number of microfilariae in 72 S. metallicum surviving 5 to 10 hr after feeding.

Shaded pillars show the flies which would have died within 24 hr, even if they were not killed.

ting the infective larvae when they have lapsed over 8 days after microfilarial ingestion at about 25°C.

As shown in Table 1, infection rates in fly groups ingesting microfilariae from upper torsos were higher than those in groups from arms or lower legs in both S. ochraceum and S. metallicum. Also, any considerable differences between infection rates of the both species could not be found. However, the development of larvae was synchronous in S. ochraceum but asynchronous in S. metallicum. In addition, in S. metallicum, malformed and/or dead larvae at first- and second-stages (especially at first-stage) often appeared, regardless of the number of ingested microfilariae (Figs. 2F, and 4). The body length of infective larvae from heads of S. metallicum was slightly shorter and a little variable than that from S. ochraceum (Fig. 5); mean length (± standard deviation) of 48 larvae from the former and of 104 larvae from the latter were 564.4 (±46.4)μm and 603.9 (±39.3)μm, respectively. These figures fairly approximate to those of Collins (1979). A tendency that the body length of "normal" third-stage larvae in the both species was reduced when a large number of larvae appeared in a single fly (Collins, 1979) was not clear (Fig. 6). Although the development of O. volvulus in S. metallicum was somewhat prevented as mentioned above, the number of third-stage larvae per fly in S. metallicum was slightly larger than that in S. ochraceum; GM (minimum-maximum) from upper torsos and lower legs were 3.8 (0-27) and 1.9 (0-27) for S. metallicum, and 2.5 (1-19) and 1.0 (1) for S. ochraceum, respectively.

For making a comparison of transmissive efficiency of the vectors, the distribution of third-stage larvae in the survived flies at 8–10th day after feeding is shown in Table 2.

Table 1 Infection of Onchocerca volvulus larvae in Simulium metallicum and S. ochraceum fed from three different body parts of volunteers at 8–10 days after ingestion of microfilariae

<table>
<thead>
<tr>
<th>Site</th>
<th>No. flies dissected</th>
<th>No. infected flies</th>
<th>% infection</th>
<th>No. of O. volvulus larvae* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mf</td>
</tr>
<tr>
<td>S. metallicum</td>
<td>Upper torso</td>
<td>54</td>
<td>26</td>
<td>48.1</td>
</tr>
<tr>
<td></td>
<td>Arms</td>
<td>22</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Lower legs</td>
<td>166</td>
<td>27</td>
<td>16.0</td>
</tr>
<tr>
<td>S. ochraceum</td>
<td>Upper torso</td>
<td>152</td>
<td>83</td>
<td>54.6</td>
</tr>
<tr>
<td></td>
<td>Arms</td>
<td>5</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Lower legs</td>
<td>20</td>
<td>2</td>
<td>10.0</td>
</tr>
</tbody>
</table>

* Those detected from S. metallicum included normal, malformed and dead larvae.

Mf: microfilariae, I, II: first- and second-stages, III: third-stage
Migration of third-stage larvae into the head of *S. metallicum* was less common than that of *S. ochraceum*. It was suggested from the above that *S. metallicum* was inferior to *S. ochraceum* in transmission of the parasite.

**Difference in the ability to transmit *O. volvulus* between the both species**

Although *S. metallicum* supported fairly well the development of *O. volvulus*, this species differed from *S. ochraceum* in survival after taking infected blood meal. Therefore, to know differences of the ability to transmit the parasite between both species, the proportion of females with infective larvae in the whole body and also in the head was calculated to all females fed on microfilaria-carriers (Table 3). The results suggested that the ability of *S. metallicum* would be about 1/3.5-1/5 of that of *S. ochraceum* in view of the survival after infection.

**Corrected results by re-identifying the experimental materials regarded as *S. metallicum* Simulium sp. I, which closely resembles**

### Table 2 Distribution of the third-stage larvae of *O. volvulus* in *S. metallicum* and *S. ochraceum* at 8-10 days after ingestion of microfilariae

<table>
<thead>
<tr>
<th>Species</th>
<th>No. flies with 3rd-stage larvae (%)</th>
<th>No. 3rd-stage larvae (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Head</td>
</tr>
<tr>
<td><em>S. meta.</em></td>
<td>41(100.0)</td>
<td>20(48.8)</td>
</tr>
<tr>
<td><em>S. ochr.</em></td>
<td>85(100.0)</td>
<td>58(68.2)</td>
</tr>
</tbody>
</table>
S. metallicum in appearance, was found by the taxonomic survey of black flies in the pilot area in the first year of our project in Guatemala (Onishi et al., 1977). The both species could be distinguished only in the larval stage, when the present study was carried out in Guatemala. Therefore, we were concerned about whether this species was involved in the specimens identified as S. metallicum, and the female genitalia of the material after dissection for parasite larvae were preserved in 70% alcohol since the midst of the experiments. Later, some identifiable points of S. sp. 1 in the adult stage were discovered, and the description

Table 3 Rate of transmissible flies surviving more than 8 days after feeding on microfilaria-carriers

<table>
<thead>
<tr>
<th>Species</th>
<th>S. metallicum</th>
<th>S. ochraceum</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of flies fed</td>
<td>330</td>
<td>196</td>
</tr>
<tr>
<td>No. of flies with infective larvae</td>
<td>41</td>
<td>85</td>
</tr>
<tr>
<td>%</td>
<td>12.4</td>
<td>43.4</td>
</tr>
<tr>
<td>No. of flies with infective larvae in the head</td>
<td>20</td>
<td>58</td>
</tr>
<tr>
<td>%</td>
<td>6.1</td>
<td>29.6</td>
</tr>
</tbody>
</table>

Table 4 Corrected results by partially re-identifying the experimental materials regarded as S. metallicum, owing to the discovery of a new member in S. metallicum-complex of Guatemala

<table>
<thead>
<tr>
<th>Species</th>
<th>S. metallicum</th>
<th>S. horacioi*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of flies dissected</td>
<td>100</td>
<td>17</td>
</tr>
<tr>
<td>Percent of flies</td>
<td>85.5</td>
<td>14.5</td>
</tr>
<tr>
<td>No. of flies with O. volvulus</td>
<td>26**</td>
<td>3**</td>
</tr>
<tr>
<td>No. of flies with infective larvae</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Percent of flies with infective larvae</td>
<td>20.0</td>
<td>5.9</td>
</tr>
<tr>
<td>No. of infective larvae</td>
<td>139</td>
<td>1</td>
</tr>
<tr>
<td>No. of flies with unknown filaria***</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>No. of flies with infective larvae</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Percent of flies with infective larvae</td>
<td>1.0</td>
<td>29.4</td>
</tr>
<tr>
<td>No. of infective larvae</td>
<td>2</td>
<td>161</td>
</tr>
</tbody>
</table>

* A new member of S. metallicum-complex reported by Okazawa and Onishi (1980).
** Some flies had only developing stage larvae indistinguishable from O. volvulus.
*** This filaria was very similar in appearance to "unknown filaria" described by Garms (1975).
of this species as *Simulium horacioi* was published in 1980 by Okazawa and Onishi who participated in this project.

The results obtained by re-identifying those preserved genitals of 117 specimens showed to include 17 (14.5%) of *S. horacioi* (Table 4). As shown obviously from the table, *O. volvulus* developed better in *S. metallicum* than in *S. horacioi*. It was suggested that *S. horacioi* bite human body and has probably a little low affinity for *O. volvulus*. Moreover, *S. horacioi* tends to be infected more frequently with "unknown filaria*" than *S. metallicum*. *S. horacioi* or *S. metallicum* might have already been infected with these unknown filaria larvae before those flies were caught.

**DISCUSSION**

Some *S. horacioi* were demonstrated among the experimental materials regarded as *S. metallicum*. Accordingly, those materials should be treated as *S. metallicum* complex, but in this paper was designated simply as "*S. metallicum*" for convenience. From the above fact it is probable that all data reported up to date as *S. metallicum* in Guatemala included *S. horacioi*.

Although wild flies of *S. metallicum* and *S. ochraceum* were used in these experiments, almost all larvae of *O. volvulus* detected from them should have derived from the experimental infections, because natural infection rates with filariae indistinguishable from *O. volvulus* in both species collected at places neighboring with the experimental collection sites in the same season were very low; the rates of the flies with third-stage, and first or second-stage larvae were 0.07% (2/2638) and 0.38% (10/2638) for *S. metallicum*, and 0.20% (2/1015) and 1.18% (12/1015) for *S. ochraceum*, respectively (Tanaka et al., unpublished).

As for the effect of microfilarial intake on the survival of flies, several investigators noted that in *S. metallicum* a high mortality was caused when more than 5-6 microfilariae were ingested (De Leon and Duke, 1966; Omar and Garms, 1977), and in *S. ochraceum* the survival was reduced to some extent by a large number of microfilariae ingested (De Leon and Duke, 1966; Collins et al., 1977). Higher survival rates were obtained in the present experiments. They were 88% even when 26.2 microfilariae were ingested in *S. metallicum*, and more than 96% regardless of the microfilarial number in *S. ochraceum*. These higher rates may be due to the fact that flies were appropriately kept under the dark and the stabilized lower temperature just from the collection.

In *S. ochraceum*, synchronous and orderly larval development was observed, as shown in previous works (De Leon and Duke, 1966; Collins et al., 1977; Collins, 1979; Matsuo et al., 1980), and also the migration of infective larvae to the head was smooth. These indicate that *S. ochraceum* is a very favorable intermediate host for *O. volvulus* and is presumed efficiently in transmission. As to *S. metallicum*, De Leon and Duke (1966) noted that only a few larvae developed to infective stage in 7-8 days at 22-27°C, and microfilariae or sausage larvae, frequently with stunted and degenerate appearance, were still present. Collins (1979) showed that the development in this species was asynchronous and slow, and the rate of third-stage larvae on days 8-10 at 19.4°C (range 16.1-25.6) was only 26.3%. On the contrary, the present studies showed that the larvae of *O. volvulus* well developed in *S. metallicum*, as indicated by the rate of third-stage larvae being 54% or 64%, and *S. metallicum* exceeded *S. ochraceum* in the number of third-stage larvae per fly. However, transmissibility of *S. metallicum* was estimated to be about 1/3.5-1/5 of that of *S. ochraceum*. The higher affinity of *S. metallicum* than in those previous reports may be caused by the fact that the experimental flies were maintained under a constant and favorable temperature, 25±0.5°C, for larval development (Matsuo et al., 1980). Nevertheless, the development of parasites was asynchronous, accompanied with many abnormal larvae. This clearly indicates that *S. metallicum* has physiological incompatibility for *O. volvulus*, to some degree, as discussed by Collins (1979). Also, weaker tendency for infective larvae
to migrate to the head of the fly may be related to the above fact. Because the development of parasites in a physiologically incompatible host is expected to be more severely affected by extrinsic low temperatures than in a compatible host, natural infection rates of _S. metallicum_ in most endemic foci of Guatemala, being always under low temperatures, would become lower than in the experiment under favorable temperatures.

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**References**


摘   要

Simulium metallicum と S. ochraceum の Onchocerca volvulus に対
する親和性の比較

Onchocerca volvulus ミクロフィラリア陽性者を吸血
した野外採集成虫を 25℃ 恒温下で飼育し, Simulium metallicum の O. v. 幼虫に対する親和性を, 好適中間
宿主 S. ochraceum と比較しながら調べた。その結果,
プユ体内での O. v. 幼虫の発育日数は, ミクロフィラリ
ア採取 7 日後に感染幼虫に達し, 8 日後にそれがプユの
頭部へ現れはじめ, 同種の間で差がなかった。しかし,
S. o. はミクロフィラリアとりこみ数に関係なく高い生
存率を示し, その体内で O. v. 幼虫がそろって感染幼虫
に発育し, 逐次的にプユ頭部へ移行した。これに対して
S. m. はミクロフィラリアのとりこみ数が増加するにつ
れて早期死亡個体が増加し, 平均 26 匹以上の摂取群で
高い死亡率を示した。また, O. v. 幼虫の発育が不ぞろ
いで, 発育遅延, 奇形や死亡虫が頻出し, S. m. はこの
寄生虫に対してある程度生理的に不適合性をもつことが
推察された。ミクロフィラリア陽性者を吸血し, 8 日以
上生存して感染可能となる雌の割合は, S. o. では 30-43%
であったが, S. m. ではその 1/3.5-1/5 程度であると推測された。
S. m. に酷似する S. horacioi は, 暫定的に S. m. と同定した実験材料の中に約 15%混在す
ることが確認され, S. m. より低くはあるが O. v. に対
して親和性をもつものと思われた。