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Laboratory observations on oviposition and egg development of Guatemalan *Simulium ochraceum* (Diptera: Simuliidae) at different temperatures[1)]

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Abstract: The oviposition and egg development of wild female *Simulium ochraceum* were observed under various constant temperature conditions (15, 18, 21 and 24°C) in the laboratory. Forty-seven (55.3%) out of 85 blood-fed females successfully oviposited in small tubes; a mean of 82.5% of their mature ova were laid. Hatching occurred in 34 (72.3%) of 47 egg batches laid. Ovulation and egg development rates were more depressed at 24°C than at lower temperatures. The rate of egg development became higher as temperature increased, with the mean time (±SD) required being 9.8±0.65, 7.2±0.27, 5.6±0.50, and 4.2±0.27 days at 15, 18, 21 and 24°C, respectively. Infection with *Onchocerca volvulus* larvae did not affect oviposition and fecundity of the fly. The fecundity correlated with body size (expressed as the cube of wing length) of the female.

INTRODUCTION

Inducement of oviposition in the laboratory is one of the key factors in the establishment of laboratory colonies of vector blackflies. Some blackfly species can be induced to oviposit by CO_2, decapitation, crushing the head or shaking the female in a tube with water (see review of Edman and Simmons, 1985). Cupp and Collins (1979) induced females of *Simulium ochraceum*, the main vector of onchocerciasis in Guatemala, to oviposit by CO_2 treatment to study their follicular relic formation. However, forced oviposition sometimes results in many unfertilized eggs. Oviposition of *S. ochraceum* in the laboratory was induced with little manipulation in a plastic tube floated in a water bath, and egg hatching was also observed (Monroy E., 1979). Detailed features of the oviposition behaviour and egg develop-

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ment of this species, however, are still unclear. Following a simple method used for *S. takahasii* (Takaoka, 1985), the present study aimed chiefly at examining oviposition and development period at the egg stage at various temperatures. Effect of *Onchocerca volvulus* infection on oviposition and fecundity was also assessed.

Materials and Methods

Wild females of *S. ochraceum* were collected on September 2, 1986, at Finca El Brote, Chicacao, Suchitepéquez, Guatemala. The flies were allowed to feed to repletion on a volunteer infected with *Onchocerca volvulus*. In total, 97 blood-fed flies were collected and maintained individually in polystyrene tubes (1.0×7.5 cm). Each fly was transferred into an oviposition tube (1.5×10.7 cm of polystyrene tube containing about 2 ml of distilled water and 1.0×7.0 cm of paper) 1.5 days after capture. The flies were supplied with squashed cotton balls soaked with a 35% sugar solution.

After 2-day maintenance at room temperature (20.1–28.5°C), the 85 survivors were divided into 4 groups of 22, 22, 21 and 20 individuals each, and placed at constant temperatures of 15, 18, 21 and 24°C, respectively. They were examined twice a day for oviposition until 7 days after the blood-feeding. Dead or moribund flies were removed and preserved in 70% ethanol for later dissection. No fly oviposited within 2 days. On the 8th day after blood-feeding, all surviving females were killed and preserved.

The eggs laid were allowed to develop in the water for 14 days. The egg batch was then fixed and preserved in 70% ethanol when some of the eggs had hatched. The number of first-instar larvae and remaining eggs were counted later. On dissection of females, residual eggs and parasite infection were examined, and wing length was measured.

Results and Discussion

1. *Oviposition method in the tube*

The eggs were laid separately on the paper beneath the water surface in the oviposition tube. Some eggs were on the side wall of the tube above the water level. The females probably laid their eggs individually in the water judging from the arrangement of the eggs on the substrate. This kind of oviposition method recalls the observation in the field by Takaoka (1981) that the female of *S. ochraceum* repeatedly hovered 10–30 cm above the surface of the water and tapped her abdomen against the surface of the water every few seconds.

The egg was subtriangular in lateral view and its size was 203.5±13.15 μm long × 125.3±7.70 μm wide (mean±SD, $n=50$). The total length and head width of a first-instar larva were 487±17.8 μm and 96±4.2 μm ($n=30$), respectively.

2. *Oviposition and egg development at various temperatures*

In this study, 47 (55.3%) of the 85 females examined successfully oviposited without any artificial encouragement (Table 1). This rate is comparable to that of Monroy E. (1979). Although the proportion of oviposited females varied from 36.4 to 71.4%, there was no difference in oviposition rates among the various temperature conditions ($\chi^2=5.585$, $df=3$, $p>0.10$). On the other hand, the overall ovulation rate (number of eggs laid/number of ova matured) in flies which oviposited at a temperature of 24°C was significantly lower than those rates in flies at other temperatures (Table 1; $\chi^2=784.5$, $df=3$, $p<0.005$). This decreased rate is attributable to the fact that half of the flies maintained at 24°C ovulated only 80% or less of their mature ova, while the other half and most of the flies at lower temperatures ovulated more than 80% (Table 2). Thus it is suggested that oviposition is adversely affected by high temperature of 24°C or more.

Similar adverse effect of high temperature was also indicated on the development of eggs laid. The overall rate of egg development at 24°C was 20.4%. This is significantly lower than those observed at other temperatures (Table 1; $\chi^2=343.3$, $df=3$, $p<0.005$). It should, however, be noted that the actual rates of egg development will be somewhat higher than those obtained because some eggs were still in earlier embryonic

Table 1 Laboratory oviposition and egg development of *Simulium ochraceum* under different temperature conditions.

	Temperature (°C)				Total
	15	18	21	24	
No. flies examined	22	22	21	20	85
No. flies oviposited	12	8	15	12	47
%	54.5	36.4	71.4	60.0	55.3
No. ova matured	2,199	1,461	2,820	1,932	8,412
No. eggs laid	1,842	1,438	2,436	1,221	6,937
%	83.8	98.4	86.4	63.2	82.5
No. eggs developed*	957	446	915	249	2,567
% of laid eggs	52.0	31.0	37.6	20.4	37.0

* No. eggs which hatched or reached at least the stage with eye spots.

Table 2 Ovulation rate of *Simulium ochraceum* in the laboratory.

Ovulation rate* (%)	No. flies				
	15	18	21	24(°C)	Total
81-100	9	8	12	6	35
61- 80	0	0	2	1	3
41- 60	1	0	0	1	2
21- 40	0	0	0	1	1
1- 20	2	0	1	3	6
Total	12	8	15	12	47

* (No. eggs laid/No. ova matured) × 100.

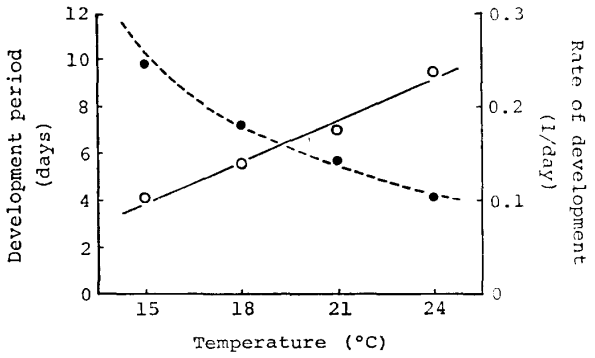

Fig. 1 Relationship between temperature, development period (in days) and rate of development (1/day) of *Simulium ochraceum* at egg stage.

Open circles, rate of development; closed circles, development period.

development, especially under lower temperatures, when observations ceased.

Hatching was observed in 34 (72.3%) out of 47 egg batches laid. First hatching was noted 9.8±0.65, 7.2±0.27, 5.6±0.50, and 4.2±0.27 (mean±SD) days after oviposition at 15, 18, 21 and 24°C, respectively (Fig. 1). Monroy E. (1979) reported that eclosion of this species was observed 5–7 days after oviposition at 20–22°C, which is comparable to the present result. The rate of egg development correlated positively with temperature. The correlation equation between the rate of development (R) and temperature (T) was calculated as follows:

$$R = 0.0149T - 0.127$$
$$(r = 0.992,\ p < 0.001).$$

Summarizing these results, a temperature of 18–21°C seems most appropriate for laboratory studies involving both oviposition and egg development since detrimental effects on both processes were apparent at 24°C, even though the time required for egg development was the shortest among the temperatures tested.

3. *Effect of* Onchocerca volvulus *infection on oviposition and fecundity*

The infection with *O. volvulus* larvae showed no effect on oviposition of *S. ochraceum*. Eight (67%) of 12 infected and 39 (54%) of 72 uninfected flies laid their eggs successfully. These proportions did not differ

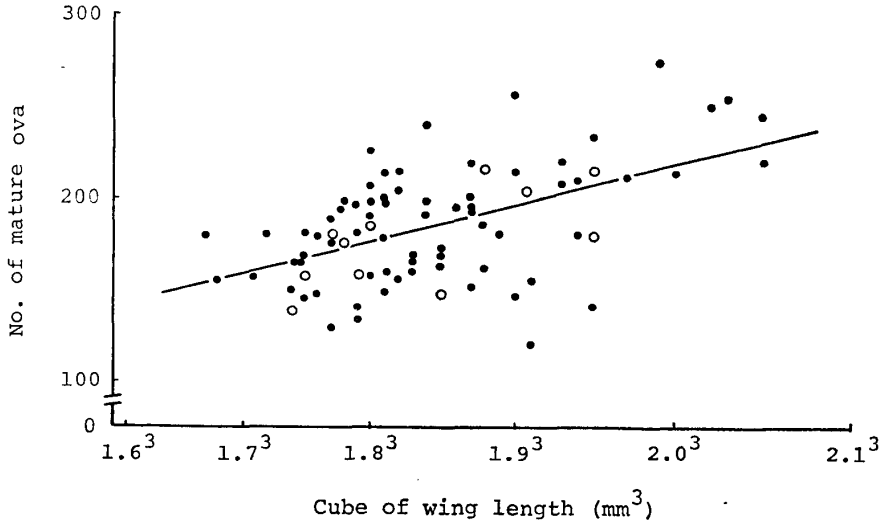

Fig. 2 Relationship between wing size and number of mature ova of *Simulium ochraceum*.

Open circles, *Onchocerca volvulus* larvae positive fly; closed circles, *O. volvulus* larvae negative fly; bold line, regression for *O. volvulus* negative flies.

statistically ($\chi^2=0.666$, $df=1$, $p>0.25$). However, it should be kept in mind that the parasite burden was low (*i.e.*, 1–5 first-stage larvae in 8 flies, 4 second-stage larvae in 1 fly and 1–3 third-stage larvae in 3 flies). Unfortunately, effect of temperature on oviposition of infected flies could not be evaluated because of an insufficient number of flies.

If the fecundity (number of mature ova) was related to the size of fly, it would be expected to vary according to a three dimensional index of size. Then, the cube of right wing length (or left wing length when the right one was not available) was used as an index of fly size. As shown in Fig. 2, the fecundity correlated positively with the size of fly as:

$$N=19.5L^3+63.9$$
$$(r=0.532,\ p<0.001),$$

where N is the number of mature ova and L is wing length. The regression was calculated for 71 parasite-free females having unbroken wing(s).

Onchocerca volvulus infection did not reduce the fecundity (Fig. 2). Parasite-free flies matured a mean of 186.5 ova ($n=72$) while infected ones did 177.7 ($n=12$). There was no statistical difference between them ($t=0.889$, $df=82$, $p>0.20$). Our result is contrary to that of Cheke *et al.* (1982) who found a reduction of fecundity of *S. damnosum s.l.* with *Onchocerca* spp. infection. Ham and Banya (1984) also found that fecundities of *S. ornatum s.l.* and *S. lineatum*, and oviposition rate of *S. lineatum* were depressed when infected by *O. lienalis* larvae. This discrepancy might be attributable to the difference in parasite burden.

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摘　要

グァテマラ産 *Simulium ochraceum* の室内産卵に関する観察

グァテマラ産オンコセルカ症媒介ブユ *Simulium ochraceum* の室内産卵および卵の発育を調べた．85頭の雌中，47頭が小試験管内で容易に産卵し，34の卵塊で孵化がみられた．産下された卵は成熟卵の82.5%であった．15, 18, 21 および 24°C の各温度条件では 24°C で産卵数・発育卵数が減少する傾向があり，卵の発育に要する日数はそれぞれの温度下で 9.8±0.65, 7.2±0.27, 5.6±0.50, 4.2±0.27 (平均±SD) であった．*Onchocerca volvulus* 幼虫感染の有無は産卵に影響せず，成熟卵数にも差はなかった．翅長を指標としたブユの大きさと成熟卵数には正の相関がみられた．