Notes on the age determination, ovariole changes and gonotrophic cycle of Simulium ochraceum in Guatemala

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Notes on the age determination, ovariole changes and gonotrophic cycle of *Simulium ochraceum* in Guatemala[1)]

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Abstract: Of the *S. ochraceum* in Guatemala, the shortest duration from emergence to blood-feeding, from blood-feeding to oviposition (both at 22°C) and from oviposition to next blood-feeding (under the field condition) were presumed to be 2 days, 5 days and 0 day, respectively. One gonotrophic cycle of this species was to be 5 days in the shortest case.

The average parous rate observed in the blackflies collected at four locations from October to December, 1977 were 48.7% for *S. ochraceum*, 41.2% for *S. metallicum*, 46.9% for *S. callidum* and 38.9% for *S. mexicanum*.

The parous rate showed diurnal change, those of which captured in the afternoon being somewhat higher.

The survival rate of *S. ochraceum* for 9 days after blood-feeding when this species become infective, was estimated at 27.3%.

INTRODUCTION

Grouping physiological ages and measuring the degree of ovariole development of a vector fly are indispensable to know its daily survival rate and the gonotrophic cycle. These knowledges are useful for understanding the population and/or the transmission dynamics and in discussing the vectorial role in the transmission of diseases.

Garms (1975) and Cupp and Collins (1979) reported the methods to determine the physiological age of *S. ochraceum*, the most important vector of human onchocerciasis in Guatemala and Mexico. The present paper deals with the physiological age based on those methods and the gonotrophic cycle of *S. ochraceum* together with the daily survival rate and the timing of the transmission of infective *O. volvulus* larvae in this blackfly

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species.

Materials and Methods

Collection of adult flies

Adult flies were obtained by the following two methods;

1) Newly emerged flies: Wild collected pupae of *S. ochraceum* were reared in the incubator at 22°C putting into a small plastic tube one by one, following the method of Matsuo *et al.* (1978) and after emergence, the adults were maintained in the same way with the sugar meal from a wad of cotton soaked in 0.5 % sugar solution until dissection.

2) Blood engorged flies: Fully engorged flies on human bait were collected individually with a small plastic tube in the field at Fca. Monica Ivoné (Chicacao), Fca. Peña Blanca (Esquintla), San Rafael Sumatán and Fca. Victoria (Yepocapa). The flies were transported back to the laboratory and maintained with the same procedure as newly emerged flies.

Dissection and observation

The flies were dissected day by day for seven days in a drop of saline solution on a slide glass under a microscope and the characteristic of follicular stages were observed. The parous rates were also studied for the flies collected in the field.

Since it seemed to be difficult to distinguish parous flies from nulliparous ones with the external characteristics, morphological changes of the ovaries were employed to distinguish by the methods reported by Duke (1968), Garms (1975) or Cupp and Collins (1979).

The developmental stages in terminal follicles were classified by modifying the Christopher's (1911) or Mer's (1936) method.

Results

The methods for determining the parous female

The methods so far reported (Duke, 1968; Garms, 1975; Cupp and Collins, 1979) were useful to distinguish parous flies from nulliparous ones, and the following characteristics were also observed.

1) In pulling the end of abdomen with needle, the ovary of parous flies can be taken out easily in comparison to that of nulliparous ones.

2) The common oviduct of parous flies is opaque with granules.

Observations on the ovariole development and the follicular stages

In the female flies kept in an incubator at 22°C immediately after emergence, the follicle developed to stage Ib on 2nd day and remained at the same stage until 9th day (Table 1). After blood-feeding, 4 days were required at 22°C for the development of the follicle at stage VI.

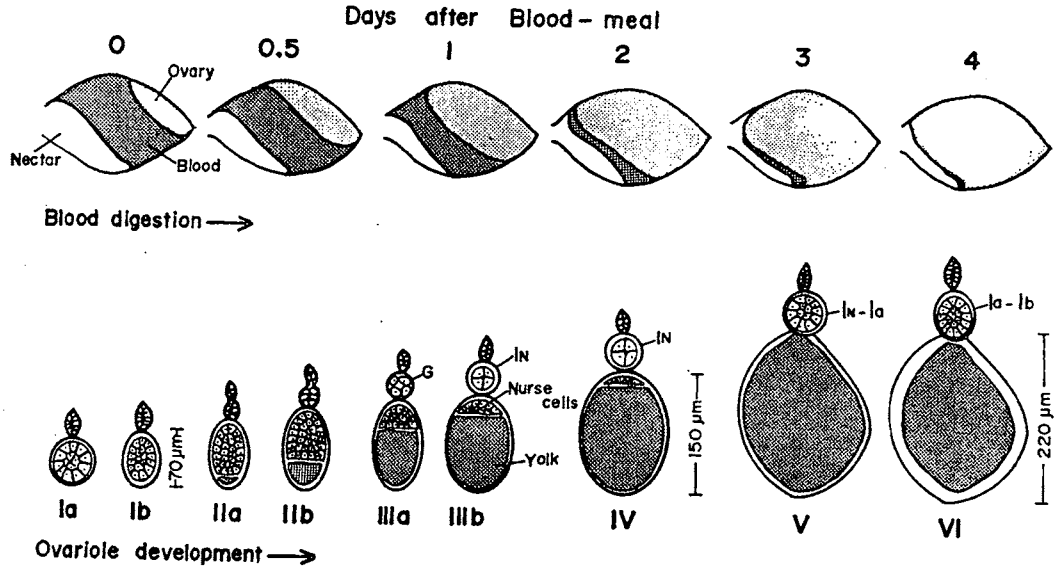

Fig. 1 Blood digestion and ovariole development of *S. ochraceum* at 22°C in laboratory

The developmental stages of ovariole after the blood-feeding were shown in Fig. 1.

Physiological age and developmental stage of the follicle in the blackflies coming to bite man in the field

The physiological conditions of *S. ochraceum* collected by human bait were shown in Table 3.

Most of the captured flies seemed to have mated at every station, showing the mating rate of 97.3% in the lowest case at Peña Blanca. The overall parous rate ranged from 35.5% to 63.2%. Although a few parous flies had apparently more than two relics in the ovarioles, the rate of multiparous could not be determined, because of the difficulty, at present, in distinguishing multiparous from uniparous clearly.

Sac-like relics were observed in those parous flies at the rate ranged from 45.6% to 78.3%. Most of *S. ochraceum* population (84.0% to 97.5%) coming to bite man in the field had follicles at stage I, although

Table 1 Development of the follicle and relationship between 1st and 2nd follicle size in the *S. ochraceum** reared in the laboratory at 22°C with sugar solution

Days after emergence	No. of flies observed	1st follicle			2nd follicle		
		Stage	Length(μm)	Width(μm)	Stage	Length(μm)	Width(μm)
1	3	IN	25-30	25-30	G	25-30	10-15
2	5	I a-I b	50-70	50-55	G	40-50	20-25
3	5	I a-I b	50-80	50-55	G	40-50	20-25
4	4	I a-I b	50-80	50-55	G	40-50	20-25
5	3	I a-I b	50-80	50-60	G	40-50	20-25
6	5	I a-I b	50-80	50-60	G	40-50	20-25
9	6	I a-I b	50-80	50-60	G	40-50	20-25

* newly adult females within 12 hr after emergence obtained from wild-caught-pupae

Table 2 Development of the follicle and relationship between 1st and 2nd follicle size in the wild caught *S. ochraceum** after blood-meal

Days after blood-meal	No. of flies observed	1st follicle			2nd follicle		
		Stage	Length(μm)	Width(μm)	Stage	Length(μm)	Width(μm)
0	5	I a-I b	50- 70	50- 60	G	40-50	15-20
0.5	3	II b	80- 90	40- 55	G	40-55	20-25
1	4	III-V	120-150	80- 90	IN	25-30**	20-30
2	4	IV-V	140-220	80-140	IN	25-30	25-30
2.5	3	V-VI	180-230	120-150	IN	25-30	25-30
3	3	V-VI	200-250	145-160	IN	25-30	25-30
4	30	VI	200-230	150-170	IN-I a	30-60	25-70
5	25	VI	200-230	150-170	IN-I a	30-70	25-70
6	27	VI	200-230	150-170	IN-I b	30-70	25-70
7	19	VI	200-230	150-170	IN-I b	30-70	25-70
8	18	VI	200-230	150-170	IN-I b	30-70	25-70
10	30	VI	200-230	160-170	IN-I b	30-70	25-70
11	39	VI	200-230	160-170	IN-I b	30-70	25-70
13	30	VI	200-230	160-170	IN-I b	30-70	25-70

* engorged females captured on man at Finca Monica Ivoné, Chicacao and Peña Blanca, Esquintla in Guatemala

** shorter length in comparison to the last observation due to split of germarium into two

Table 3 Percentage distribution of age and developmental stage of the 1st follicle in unfed females of *S. ochraceum* coming to bite man from October to December, 1977

Location	Day of collection	No. of flies dissected	Mating* (%)	Parous* (%)	Sac-** like relic (%)	Re-** sidual eggs (%)	Follicle stage* (%) IN	I	II	III	Fat-* body (%)	Nectar* sucking (%)	Average No. of ovariole (one-side)	Parasites*** (numbers observed)
Monica Ivoné	10, Oct.	100	100	39.0	64.1	0	0	84.0	16.0	0	3.0	93.0	—	F 1
	16–17, Nov.	1375	99.8	47.7	57.7	4.1	0.3	87.5	12.1	0.1	7.0	97.1	60.0	F 30, M 2
	21–22, Nov.	310	100	35.5	47.3	8.2	0	94.2	5.5	0.3	14.5	99.5	58.0	F 8
	28–29, Nov.	800	99.9	58.1	45.6	4.7	0.1	86.4	13.4	0.1	6.3	98.4	61.7	F 21
	13–15, Dec.	200	100	42.0	50.0	3.6	0	97.5	2.5	0	9.5	95.4	58.6	0
Peña Blanca	9–10, Nov.	199	99.0	44.2	47.7	5.7	0.5	63.8	34.2	1.5	5.0	79.4	67.6	F 1
	6– 7, Dec.	73	97.3	63.0	78.3	0	1.4	83.6	15.1	0	6.8	97.3	73.2	0
Victoria	25, Oct.	19	100	63.2	66.7	0	0	89.5	10.5	0	5.3	—	—	—
Guachipilín	28, Oct.	10	100	40.2	50.0	0	0	70.0	30.0	0	10.0	100	60.9	0
	Total	3086	99.8	48.7	53.0	4.7	0.2	86.7	12.8	0.2	7.5	96.3	59.9	F 61, M 2

* per total numbers examined ** per parous flies *** F=fungi, M=mites

the rate was somewhat lower in those collected at Peña Blanca (68.8%) and Guachipilín (70%).

Fat-bodies were found in most of the laboratory-bred young flies within 4 days after emergence, while they were found in only 14.6% of the wild caught nulliparous flies.

Table 4 shows the physiological conditions of the other three anthropophilic species collected at four sites. *S. callidum* exhibited the highest parous rate of 46.4% followed by *S. metallicum* (41.2%) and *S. mexicanum* (38.9%).

In general, the parous rates of the flies collected in the morning were lower than those coming to bite man in the afternoon (Figs. 2, 3).

DISCUSSION

Morphological changes of ovaries were commonly used in determining the physiological age of a certain insect. Existence of follicular relics or follicular dilatations as observed in mosquitoes (Detinova, 1962; *etc.*) could be a useful indicator also for the blackflies, but the subdivision of age was much more difficult in parous flies of this species than that of mosquitoes.

The disappearance process of the fat bodies which were observed by Crosskey (1958) and Duke (1968) in *S. damnosum* in Africa was not utilized for the present experiment, because in the case of *S. ochraceum*, the fat bodies were found only in newly emerged adults within 4 days after emergence. This fact, however, suggests that the newly emerged flies can be easily distinguished from the older ones.

The parous rates observed in the afternoon were higher than those in the morning, as well as the rate of the flies with sac-like relics. These facts indicate that the majority of parous flies would come to bite man soon after oviposition. In other words, flies with larger sac-like relics captured in the afternoon might have laid eggs in the morning on the same day, and flies with smaller sac-like relics captured in the morning might have laid eggs in the afternoon the day before (in general, 24 hr are required after

Table 4 Percentage distribution of age and developmental stage of the follicle in unfed females of three Guatemalan blackfly species coming to bite man from October to December, 1977

Species[1]	Location	Day of collection	No. of flies dissected	Mating (%)	Parous[2] (%)	Sac-like relic[3] (%)	Residual eggs[3] (%)	Follicle stage[2] (%) IN	I	II	III	Fat-body[2] (%)	Nectar sucking[2] (%)	Average No. of ovariole (one-side)	Parasites[4] (numbers observed)
met.	Sumatám	26, Oct.	31	100	41.9	23.1	0	0	96.8	0	3.2	12.9	—	—	N 1
	Guachipilín	28, Oct.	34	100	47.1	62.5	0	0	91.2	8.8	0	5.9	100	89.5	0
	Peña Blanca	8, Nov.[5]	33	97.0	36.4	25.0	8.3	3.0	66.7	30.3	0	15.2	75.8	82.3	N 1
		9–10, Nov.	31	93.5	38.7	33.3	0	6.5	71.0	22.6	0	16.1	81.5	86.5	N 2
		6– 7, Dec.	65	90.8	41.5	55.6	25.9	10.9	71.9	17.2	0	3.1	83.1	88.1	F1, M3, N 6
		Total	194	95.4	41.3	43.8	10.0	5.2	78.2	16.1	0.5	9.3	84.9	86.8	F1, M3, N10
cal.	Guachipilín	28, Oct.	7	100	42.9	66.7	0	0	100	0	0	0	100	82.6	0
	Peña Blanca	8, Nov.[5]	39	100	53.8	23.8	0	0	82.0	15.4	2.6	43.6	89.7	94.3	M1
		9–10, Nov.	37	93.7	56.7	52.4	0	2.7	81.1	16.2	0	37.8	73.0	91.6	F1, M0, N 3
		6– 7, Dec.	30	93.3	26.7	87.5	12.5	3.3	86.7	6.7	3.3[6]	13.3	73.3	97.0	M1, N 1
		Total	113	97.3	44.2	47.2	1.9	1.8	84.1	12.4	1.8	31.0	80.5	93.7	F1, M2, N 4
mex.	Peña Blanca	8, Nov.[5]	18	100	38.9	57.1	0	0	50.0	44.4	5.6	0	66.7	129.2	F0, M0, N 0

[1] met.=*Simulium metallicum*, cal.=*S. callidum*, mex.=*S(H). mexicanum*
[2] per total numbers examined
[3] per parous flies
[4] F=fungi, M=mites, N=nematodes
[5] collected on horse
[6] stage V follicle

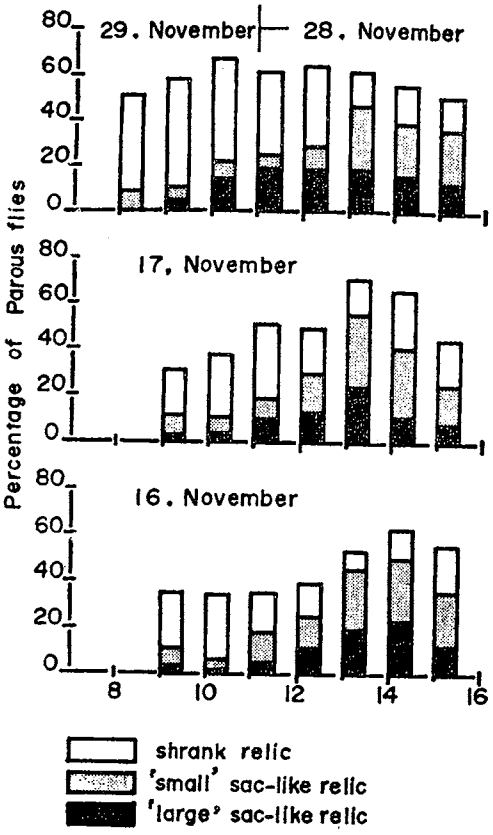

Fig. 2 Diurnal changes of parous rate and sac-like relic of *S. ochraceum* at Finca Monica Ivoné, Chicacao in 1977

species in Guatemala (Garms, 1975; Garms and Ochoa, 1979).

A sufficient number of flies could not be obtained on the 21st and 22nd, November 1977. However, the rate of the flies with fat body was high and the parous rate was low. This suggests that a number of fresh adults had emerged around these days (Fig. 3).

As shown in Table 2, flies which showed the fastest ovariole development seem to mate and take the first blood meal the day after emergence.

Four days were required for the maturation of ovarium in engorged female in the shortest case, and the deposition of eggs may take place next day. Then the 2nd blood meal may be taken on the same day. Thus, one gonotrophic cycle, in the shortest case, would be 5 days in this study, while Garms and Ochoa (1979) reported that the cycle would be 2 to 4 days for this species. But if the day of oviposition was not calculated as 1 day of the next cycle as we did, their results on the gonotrophic cycle could be 3 to 5 days.

According to the other laboratory studies, development into infective larvae from micro-

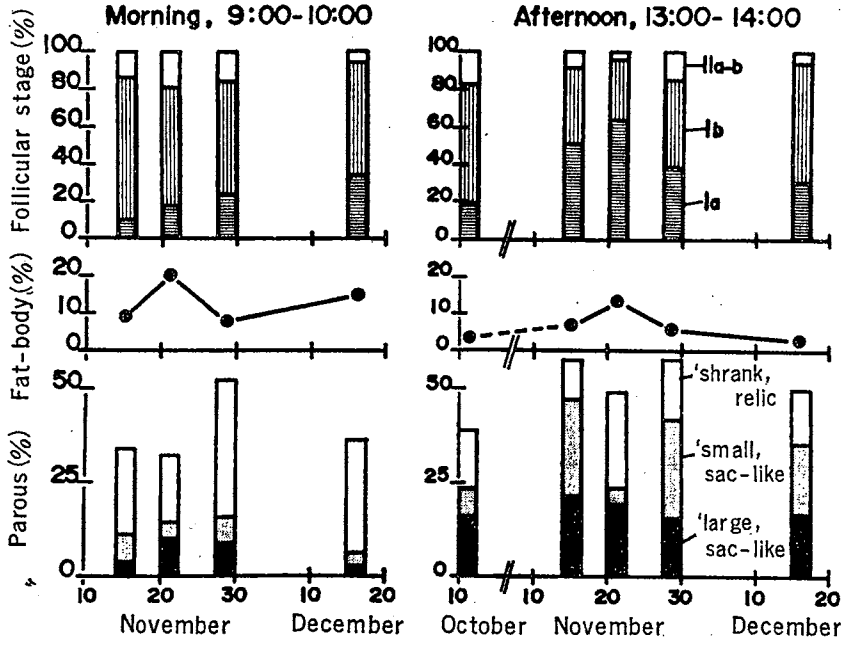

Fig. 3 Monthly changes of parous rate, fat-body and follicular stage of *S. ochraceum* at Finca Monica Ivoné, Chicacao in 1977

oviposition for the large sac-like relic to shrink (Detinova, 1962; *etc.*)). The same phenomena were already recorded in this

filariae in *S. ochraceum* was to require 8 days at 25°C (Matsuo *et al.*, 1980), 7 to 8 days at 22 to 27°C (DeLeon and Duke, 1966)

Table 5 Presumptive relationship between gonotrophic cycle of *Simulium ochraceum* and transmission of *Onchocerca volvulus* from laboratory experiments

Days after emergence	*Simulium ochraceum*			*Onchocerca volvulus*	
	Gonotrophic cycle (shortest case)	Survival rate after blood-feeding*	Days after blood-feeding	Development of Mf. in *S. ochraceum* at 25°C**	30°C***
1	emergence				
2	mating?				
3	1st blood feeding	100%	0	Mf. taken by *S. ochraceum*	
4	1st gonotrophic cycle (5 days)	86.6	1		
5		75.0	2		develop to infective larvae
6		64.9	3		
7		56.2	4		larvae migrate to head
8	1st oviposition and 2nd blood feeding	48.7	5 0	develop to infective larvae	become transmissible
9	2nd gonot. cycle	42.2	6 1		
10		36.4	7 2		
11		31.5	8 3	larvae migrate to head	
12		27.3	9 4	become transmissible	
13	2nd oviposition and 3rd blood feeding	23.7	10 5 0		
14	3rd gonot. cycle	20.5	11 6 1		
15		17.8	12 7 2		
16		15.4	13 8 3		
17		13.3	14 9 4		
18	3rd oviposition and 4th blood feeding	11.6	15 10 5		
19	4th gonot. cycle	10.0	16 11 6		
20		8.7	17 12 7		
21		7.5	18 13 8		
22		6.5	19 14 9		
23	4th oviposition and 5th blood feeding	5.6	20 15 10		

* From the Tables 1 and 2, the duration of one gonotrophic cycle of *S. ochraceum* is 5 days and average parous rate is 48.7%, hence the daily survival rate is 0.866 (Davidson, 1955).

** Matsuo *et al.* (1980) *** Collins (1977)

and 4 days at 30°C (Collins, 1977). Assuming that the average atomospheric temperature in the endemic areas in Guatemala is 20 to 25°C, 8 days seem to be needed for the microfilariae to become infective larvae under the field conditions in this country. This means that *S. ochraceum* can transmit the infective larvae of *O. volvulus* at the 3rd or the later blood meal, in other words, 9 days or more after blood feeding (Table 5).

Daily survival rate is also estimated from the results mentioned above using the formula used for mosquito studies (Davidson, 1955; Wada, 1975). Since the average parous rate of *S. ochraceum* was 0.487, and one gonotrophic cycle was assumed to be 5 days at 22°C (average atomospheric temperature in the endemic area in Guatemala), the daily survival rate is 0.866. Therefore, the survival rate on the 9th day after blood feeding was $0.866^9=0.273$ (Table 5).

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摘　要

グァテマラにおける *Simulium ochraceum* の生理的年齢，卵巣小管の発育変化および吸血間隔 (gonotrophic cycle) の観察

室内および野外個体の解剖観察をもとに，*S. ochraceum* の羽化から吸血までの最短期間を2日，吸血から産卵までを5日，産卵後まもなく2回目の吸血をすると仮定すると，吸血-産卵-吸血 (gonotrophic cycle) の最短期間は5日と考えられた．

調査4地点（1977年10月～12月）の平均産卵経験率は *S. ochraceum* 48.7%，*S. callidum* 46.9%，*S. metallicum* 41.2%，*S. mexicanum* 38.9%であった．産卵経験率および sac-like relic 率は日変化を示し，午後に高い傾向を示した．

以上のことから *S. ochraceum* の日生存率は0.866と計算され，羽化後1回目の吸血で *O. volvulus* の Mf. を取り込んだとして，これが感染型に発育し，伝播可能になる吸血後9日目の生存率は27.3%と推定された．