# LABORATORY OBSERVATIONS ON THE BIOLOGY OF THE PHLEBOTOMID SANDFLY, *PHLEBOTOMUS PAPATASI* (SCOPOLI, 1786)

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**Abstract.** Investigations on the biology of *Phlebotomus papatasi* were carried out under laboratory conditions at 28 ± 2°C and 80 ± 1% RH. Fecundity of the female varied between 61 and 48 (mean 56.2 ± 5.46) and the incubation period of eggs ranged from 7-9 (mean 7.81 ± 0.61) days. The developmental duration of larvae and pupae varied from 24 to 31 (mean 28.57 ± 2.71) and 9 to 11 (mean 10.38 ± 1.51) days, respectively. The rate of insemination, determined from the females that laid fertile eggs, was found to be maximum when the females were three days old. Autogeny was observed from female sandflies, emerged from the larvae fed on animal liver powder. The duration of first and second gonotrophic cycles under ambient conditions was shortest in summer and longest in winter. Studies on adult longevity showed that the females fed on mouse blood survived for 6 to 27 days (mean 14 ± 12.5 days).

## INTRODUCTION

*Phlebotomus papatasi,* the vector of leishmaniasis, has a wide distribution in Old World and has been reported to transmit Kala-azar in Malta (Cachia abd French, 1964), infantile Kala-azar in Iraq (Sukkar *et al,* 1982) and cutaneous leishmaniasis in India (Farooq and Qutubudin, 1945). Natural infection with leptomonad forms was also reported from *P. papatasi* from Kala-azar endemic area of Muzzaffarpur, Bihar, India (Dhanda *et al,* 1983). Though extensive literature is available on the ecology of this species (Dhanda *et al,* 1983; Dhanda and Gill 1982; Joshi *et al,* 1979) very little is known about its biology due to the difficulties in studying the immatures in nature as the breeding places are difficult to locate. In this context an in-depth understanding on such aspect is necessary in devising any control strategy. The present communication reports a study on the biology of both immatures and adults of this species under laboratory conditions.

## MATERIALS AND METHODS

To determine the fecundity, incubation period and hatchability of eggs, twenty-five freshly emerged and mated females obtained from a cohort of eggs were fed on mouse blood according to the method described by Srinivasan *et al* (1992). These were then confined individually in 250 ml plastic container having moist plaster of paris at the bottom and corrugated filter paper (Whatman No. 1). The females were given 50% honey and observed daily for oviposition. The number of eggs laid were recorded and allowed to hatch in the same containers and the larvae were reared on a diet consisting of powdered fecal pellets of rabbit and Bentonite, a dehydrant. The developmental duration and survival rate of immatures were also calculated from the same batches of experiments. The immature survival rate was calculated from the proportion of larvae successfully developed into adults. Influence of larval diet such as fecal pellets of the house rat, mouse and rabbit and a mixture of fecal pellet and animal liver powder on autogeny was also investigated.

To study the mating behavior batches of ten females, each 24, 48, 72, 96 and 120 hours of age were exposed to 48 hour old males for a period of 24 hours. Due to the difficulty in examining the presence of sperm in the spermathecae, the inseminated females were identified indirectly from the fertile eggs. After exposure to males, the females were fed on mouse blood. The fed females were then confined to individual containers for oviposition. The eggs thus obtained were allowed hatch and the rate of insemination rate estimated on the basis of batches in which hatching was observed. The experiment was replicated five time. Throughout the period of study the ambient temperature

and humidity were maintained at 28 ± 2°C and RH 80 ± 1%, respectively.

The duration of first and subsequent gonotrophic cycles under simulated field conditions was studied by releasing 100 freshly emerged females along with an equal number of males of the same cohort in a cloth cage (30 × 30 × 30 cm) and was provided with 50% honey solution. The females were fed on mouse blood and then provided ovitraps for egg laying. The ovitraps consisted of cones made of Whatman no. 1 filter paper kept in petri-dishes containing little tap water. The experiment was continued until the last female died. The duration of gonotrophic cycle was determined from the peak oviposition day.

To find out the oviposition periodicity, five gravid females were released individually into 25 ml test tubes having moist filter paper. These females were observed under dissection microscope at three hour interval from 18.00 hours to 06.00 hours and the number of eggs laid were counted.

## RESULTS AND DISCUSSION

The females laid the eggs singly on moist surface. The number of eggs laid by a female varied from 48 to 61 with a mean of 56.2 ± 5.46 eggs. The incubation period of eggs of a single cohort ranged from 7 to 9 days (mean 7.81 ± 0.61 days) and hatchability scored in an average was 92.8% with a range from 83.3 to 98.5%. The developmental duration of larvae varied between 24 and 31 (mean 28.57 ± 2.71) and that of pupae between 9 and 11 (mean 10.38 ± 1.51) days (Table 1). The rate of pupation varied from 59.5 to 95.9% (av 79.9%) and survival rate of immatures from 58.7 to 93.8% (av 87.8%). The proportion of males and females emerged was 52.7% and 47.3%, respectively, with a male to female ratio of 1.0 : 0.9.

Majority of the adults were seen to involve in mating on the second day of emergence and the activity lasted for 25 to 52 seconds (mean 31 ± 7.28 seconds). The rate of insemination as determined from the females that laid fertile eggs, in each of the age groups *viz,* 24, 48, 72, 96 and 120 hours old, was 8%, 28%, 84%, 44% and 36% respectively. It was maximum on day 3 and was significantly higher ($p < 0.05$) as compared with other females.

Table 1

Developmental duration of immatures of *P. papatasi* (28 ± 2°C and RH 80 ± 1%).

| Stage of development | Developmental duration (in days) | |
|---|---|---|
| | Range | Mean ± SD |
| Eggs (N = 100): | | |
| | 7 - 9 | 7.81 ± 0.61 |
| Larva (N = 100): | | |
| I | 6 - 7 | 6.99 ± 1.16 |
| II | 4 - 5 | 4.00 ± 0.50 |
| III | 5 - 6 | 4.88 ± 0.64 |
| IV | 9 - 13 | 10.50 ± 1.64 |
| Pupa: | 9 - 11 | 10.38 ± 1.51 |

The percentage of females engorged on mouse blood was 0, 3.33, 57.33, 24.89, 8.68 and 2.44 on 1st, 2nd, 3rd, 4th, 5th and 6th day of emergence respectively. The remaining females (3.33%) died without feeding. The proportion of females that fed on the 3rd day was significantly higher ($p < 0.05$) than those that fed on other days. The duration required for a complete blood meal was 3 to 8 minutes (mean 6.11 ± 1.52). These finding were similar to those observed by Gemetchu (1976) for *Phlebotomus longipes.* The majority of the females laid viable eggs after a single blood meal. The process of blood digestion and development of ovary was also found to occur simultaneously, which indicates that *P. papatasi* is gonotrophically concordant and also confirm the findings of Dolmatova (1942). However, Killick-Kendrick (1983) and Schmidt and Schmidt (1965) reported that *P. papatasi* needed more than one blood meal during a gonotrophic cycle.

The duration of gonotrophic cycle varied according to season. It was 8 to 5 days during summer, 9 to 6 days in monsoon and 11 to 8 days in winter (Table 2). Heavy mortality of females was observed after first gonotrophic cycle in all the seasons, only a few females survived for second oviposition and none survived thereafter.

Females laid the maximum number of eggs in the third quarter of the night (Table 3) and which was significantly higher ($p < 0.05$) from the number of eggs laid in remaining quarters of the night.

Table 2

Number of eggs laid by *P. papatasi* on different days during different seasons.

| Duration after emergence | Number of eggs laid | | |
|---|---|---|---|
| | Summer | Monsoon | Winter |
| Day 1-6 | - | - | - |
| 7 | 39 | - | - |
| 8 | 424 | - | - |
| 9 | 17 | 356 | - |
| 10 | 3 | 37 | 7 |
| 11 | - | - | 347 |
| 12 | - | - | - |
| 13 | - | - | - |
| 14 | 30 | - | - |
| 15 | - | - | - |
| 16 | - | 17 | - |
| 17 | - | - | - |
| 18 | - | - | - |
| 19 | - | - | 24 |
| 20 | - | - | - |

summer = March - June,
monsoon = July - October,
winter = November - February.

Table 3

Oviposition periodicity of *P. papatasi.*

| Quarters of night | Duration in hour | Number of eggs deposited | |
|---|---|---|---|
| | | Range | Mean (SD) |
| I | 18.00-21.00 | 3-12 | 7.6± 3.44 |
| II | 21.00-24.00 | 15-37 | 26.6± 8.59 |
| III | 24.00-03.00 | 71-117 | 91.8±18.26 |
| IV | 03.00-06.00 | 2-21 | 10.2± 6.31 |

It was observed that, three out of 10 females that emerged from the larvae fed on animal liver powder laid a total of 17 eggs without a blood meal. This shows that the females are autogenous. Dolmatova (1946) also reported similar such phenomenon in this species. The number of eggs laid by an autogenous female varied from 3 to 9 (av 5.67 eggs). All these eggs hatched to larvae. The females obtained from the immatures fed on food materials other than animal liver powder failed to produce eggs. This observation shows that a high protein diet like animal liver powder induces autogeny (1970).

Studies on adult longevity showed that the males and females fed on 50% honey solution survived from 14 to 28 days (mean 19±2.7 days) and 7 to 31 days (mean 12±5.27 days) respectively. When the adults were maintained on soaked raisins, males survived from 11 to 19 days (mean 16±2.6 days) and the females from 9 to 29 days (mean 15±7.25 days). Females fed on mouse blood survived for 6 to 27 days (mean 14±12.5 days). Similar survival rates have earlier been reported under natural condition also (Srinivasan and Panicker, 1992).

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