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BIOLOGY OF *PHLEBOTOMUS ARGENTIPES* ANNANDALE AND BRUNETTI AND *P. PAPATASI* (SCOPOLI) IN THE LABORATORY

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SUMMARY

Laboratory breeding and productivity of a colony of *Phlebotomus papatasi* (Scopoli) over 14 generations are reported and the duration of developmental stages at different temperatures are indicated. It was found that the larvae grew better at 29°-30° C, while adult longevity and feeding activity were higher at 24°-26° C and 27 ± 0.5° C respectively. The development time from egg to adult was 39 days. The behaviour of the larvae and the detailed biology of the adults of *P. papatasi* and *P. argentipes* viz., egg laying capacity, feeding activity on laboratory animals and man, effect of aging on feeding activity, time required to respond to host stimuli, quantity of blood meal taken, multiple feeding and oviposition survival etc., are described and compared.

RÉSUMÉ : **Biologie expérimentale au laboratoire de *Phlebotomus argentipes* Annandale et Brunetti et de *P. papatasi* (Scopoli).**

Exposé des données obtenues sur la reproduction et la productivité au laboratoire d'une colonie de *Phlebotomus papatasi* (Scopoli) pendant 14 générations, ainsi que sur la durée des stades de développement suivant les températures. Le développement de la larve est meilleur à 29°-30°, alors que la longévité de l'adulte et l'activité d'alimentation sont supérieurs respectivement à 24°-26° et à 27 ± 0,05°. Les comportements des larves et des adultes sont décrits et comparés chez *P. papatasi* et *P. argentipes* en particulier : la capacité de ponte, l'activité d'alimentation sur les animaux de laboratoire et l'homme, l'effet de l'âge sur l'activité d'alimentation, le temps nécessaire pour répondre au stimulus de l'hôte, le volume du repas sanguin, les repas multiples, la survie de la ponte, etc.

INTRODUCTION

Phlebotomus argentipes Annandale and Brunetti and *P. papatasi* (Scopoli) are the two predominant Indian phlebotomine sanflies. *Phlebotomus argentipes* is widely distributed in the two highly kala-azar (visceral leishmaniasis) endemic states, Bihar and West Bengal and *P. papatasi* is found in visceral leishmaniasis (VL) endemic state of Bihar and also in cutaneous leishmaniasis (CL) endemic state of Rajasthan. It has been reported that *P. argentipes* and *P. salehi* are the incriminating vectors of *Leishmania donovani* (Shortt *et al.*, 1931; Swaminath *et al.*, 1942) and *L. major* (Kalra and Lewis, 1976; Le Blancq *et al.*, 1986) respectively in India. However, *P. papatasi* is a proven vector of *L. major* in many places (Killick-Kendrick *et al.*, 1985; Ward, 1985) and, inspite of the lack of evidence, is suspected to play some role in the transmission of *L. donovani* in India (Modi *et al.*, 1980; Sanyal, 1985). Investigations on *P. argentipes* and *P. papatasi* have been carried out by many earlier workers, although the biology of these two phlebotomines have been studied principally in the field (Napier and Smith, 1926; Smith, 1959; Das *et al.*, 1976; Pandya and Neogi, 1980; Dhiman *et al.*, 1983; Pandya, 1985; Mukhopadhyay and Chakravarty, 1987).

Colonization of *P. argentipes* (Smith, 1925; Christophers *et al.*, 1926; Shortt *et al.*, 1926; Eldridge *et al.*, 1963; Das and Mukherjee, 1969; Pandya, 1980) and *P. papatasi* (Waterston, 1922; Whittingham and Rook, 1922; Unsworth and Gordon, 1946; Eldridge *et al.*, 1963; Hafez and Zein-el-Dine, 1964; Schmidt, 1964; Pandya, 1980) has been frequently accomplished with observations made on several aspects of their biology in the laboratory. Modi and Tesh (1983) described an useful, simple technique for mass rearing of *P. papatasi* and *Lutzomyia longipalpis* in the laboratory. These authors, however, did not report the detailed biology of the laboratory colonies and, inspite of the fact that these are the best studied sandflies in the laboratory, many facets of their behaviour remain unknown or poorly understood (Ward, 1985). This paper reports details of the biology of *P. argentipes* and *P. papatasi* from India in laboratory conditions.

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MATERIALS AND METHODS

The colonies of the two phlebotomines, *P. argentipes* and *P. papatasi,* were established with wild bloodfed females from West Bengal and Bihar respectively. Modi and Tesh's (1983) method was followed for the laboratory colonization of *P. argentipes* with modifications (Ghosh and Bhattacharya, 1989). However, further modification of the temperature on different phases of rearing for *P. papatasi* were made *(Fig. 1)* because the temperature tolerance of *P. argentipes* and *P. papatasi* was not same, as evidenced by their distribution in India.

Phlebotomus papatasi was allowed to feed at ambient temperature 27 ± 0.5° C following our earlier methods on *P. argentipes.* The larval rearing pot and larval food were made as stated by Ghosh and Bhattacharya (1989). The emerged adults were kept in adult holding cages (Modi and Tesh, 1983). To have subsequent generations the emerged flies were allowed to take blood meals on white mice, hamsters, guineapigs and albino rats. However, sometimes the flies were fed on the forearm of the senior author (KNG).

After feeding on a human, skin reactions were noted for both the species. The generation time and the effect of temperature on different phases of rearing of both the species were noticed. Both species were allowed to refeed after oviposition to see their relative oviposition survival and life expectancy. Flies of different ages in separate batches were allowed to feed on the same host animal to note the time taken by different age groups to respond to host stimuli and the relative effect of aging on feeding activity. About 200 females were exposed to laboratory animals during feeding and, in order to note the effect of aging on the feeding activity and pre-biting period, about 100 females were exposed to human hands in each trial. They were kept on 30 % sucrose solution before and after blood feeding.

The weight of the blood meal was calculated by substracting the average weight of an unfed female from the average weight of a bloodfed fly in multiple batches (weighed with a Mettler AC 100 balance).

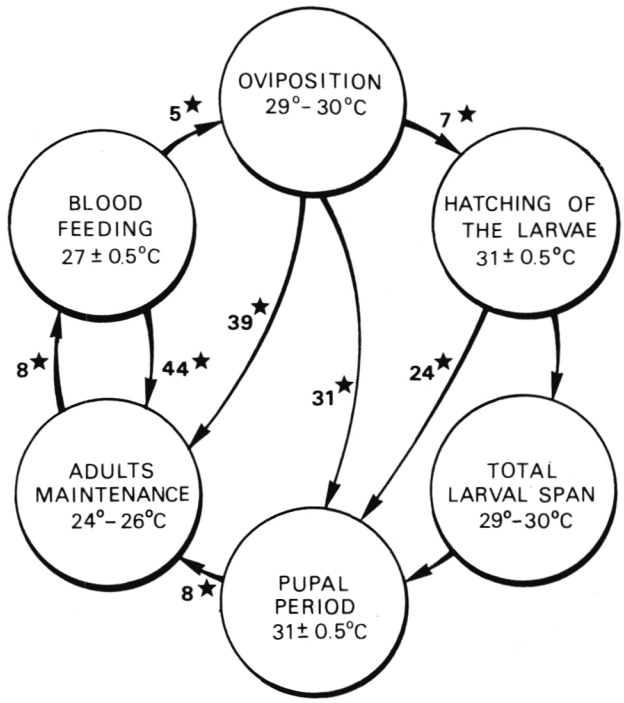

FIG. 1. — Different steps of laboratory colonization of *P. papatasi* with temperature maintained and days required in each stage. * Indicates mean days.

RESULTS

The preliminary results of the laboratory colonization of *P. argentipes* were described earlier (Ghosh and Bhattacharya, 1989). *Figure 1* shows the different steps of laboratory rearing and temperature maintained during colonization of *P. papatasi* which is now in the 15th generation. It also indicates the time taken for each stage and the total life span during development from egg to adult. The time required for each generation (egg to adult) is about 39 days *(Fig. 1)*. The effects of temperature on the biology in different phases of rearing of *P. papatasi* are shown in *Table I.*

TABLE I. — *Effect of temperature on different developmental stages and productivity of* P. papatasi *during its laboratory colonization.*

Laboratory temperature (°C)	*% of engorgement**§	*% of ovipositied flies**	*Death before oviposition* (%)*	*% not ovipositied**	*Hatching time* (days)*	*Eggs lost* (%)*	*Larval period* (days)*	*Larval loss* (%)*	*Pupal period* (days)*	*Pupal loss* (%)*	*Adult longevity* (days)*
24-26	87 (80-93)	49 (42-55)	12 (8-16)	39 (32-45)	12 (10-15)	12 (9-16)	40 (35-44)	13 (8-17)	13 (10-16)	9 (7-11)	21 (15-27)
27 ± 0.5	91 (85-96)	63 (57-69)	9 (6-13)	28 (25-33)	10 (8-12)	9 (6-13)	31 (27-35)	11 (7-15)	10 (7-13)	3 (1-5)	15 (12-18)
29-30	35 (29-40)	87 (85-93)	7 (4-11)	6 (3-10)	8 (7-10)	9 (5-13)	24 (21-27)	8 (4-12)	9 (7-11)	2 (1-4)	11 (8-14)
31 ± 0.5	13 (9-18)	68 (62-73)	23 (17-30)	9 (5-13)	7 (6-8)	10 (6-14)	21 (18-24)	13 (10-16)	8 (6-10)	3 (1-6)	5 (3-8)
32-33	5 (2-9)	46 (40-52)	51 (47-55)	3 (1-6)	6 (5-7)	31 (25-37)	18 (15-21)	23 (19-28)	6 (4-8)	12 (7-17)	2 (1-4)

§: Overnight exposure on white mice. *: Mean. Figures in the parentheses indicate the ranges.

TABLE II. — *Statistical analysis of Table I (only relevants are given).*

Observation	*Temperature group*	*Observed value of statistics*	*P value*	*Remarks*
Engorgement	A – B	$z = 2.12$	$P < 0.05$	Significant
Oviposited flies	C – D	$z = 7.19$	$P < 0.01$	Significant
Hatching time	B – C	$t = 2.58$	$P < 0.05$	Significant
	C – D	$t = 1.18$	$P > 0.05$	Insignificant
Eggs lost	C – D	$z = 0.69$	$P > 0.05$	Insignificant
	D – E	$z = 3.67$	$P < 0.01$	Significant
Larval period	C – D	$t = 2.80$	$P < 0.05$	Significant
	D – E	$t = 2.80$	$P < 0.05$	Significant
Larval loss	C – D	$z = 2.57$	$P < 0.01$	Significant
	D – E	$z = 1.84$	$P < 0.05$	Significant
Pupal period	C – D	$t = 1.11$	$P > 0.05$	Insignificant
	D – E	$t = 2.47$	$P < 0.05$	Significant
Pupal loss	C – D	$z = 1.01$	$P > 0.05$	Insignificant
	D – E	$z = 2.41$	$P < 0.01$	Significant
Adult longevity	A – B	$t = 3.91$	$P < 0.01$	Significant

A = 24°-26° C; B = 27 ± 0.5° C; C = 29°-30° C; D = 31 ± 0.5° C and E = 32°-33° C.

[To test the hypothesis HO : $P_1 = P_2$
against $H_1 > P_2$, where P_i is the proportion for ith population $i = 1,2$.

We use the statistic, $$Z = \frac{\frac{s_1}{n_1} - \frac{s_2}{n_2}}{\sqrt{\tilde{p}(1 - \tilde{p})\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

which has a standard normal distribution

where

$\tilde{p} = \frac{s_1 + s_2}{n_1 + n_2}$;

s_i = successful no. in i, $i = 1,2$;

n_i = no. of exposure in i, $i = 1,2$;

Ho is rejected (at 5 % level of significance) if $z > 1.64$.

To test Ho : $\mu_1 = \mu_2$ against $H_1 : \mu_1 > \mu_2$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

$\bar{x}_i$ = Mean successful no. in i, $i = 1,2$

$$s^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}$$

s_i = Standard deviation in ith expt. $i = 1,2$

the difference is significant if $t > t_{n_1+n_2-2,\alpha}$ $\alpha = 0.05$ or 0.01].

The percentages of engorgement by *P. argentipes* and *P. papatasi* on different laboratory animals and human are indicated in *Table III.* It was found that, as the age of the two species advanced, their engorgement rate also increased *(Fig. 2).* The pre-biting period necessary to respond to host stimuli (man) is shown in *Table IV.* It indicates that, as the age of the vector increases, the readiness of the flies to take blood meal also increases *i. e.*, pre-biting period decreases.

Table V summarises the results of multiple feeding of seven large batches of the two species. A total of 657 *P. argentipes* and 514 *P. papatasi* were offered feeds of which 543 (82.64 %) and 473 (92.02 %) respectively took a first blood meal. It was found that 400 (73.66 %) of the former and 403 (85.20 %) of the latter oviposited. A total of 61 (11.23 %) and 131 (27.69 %) of *P. argentipes* and *P. papatasi* took a 2nd blood meal after 186 (34.25 %) and 276 (58.35 %) survived 1st oviposition respectively. It was also found that 22 (4.05 %) of *P. argentipes* and 64 (13.53 %) of *P. papatasi* oviposited for the 2nd time and 8 (1.47 %) and 31 (6.55 %) survived a 2nd oviposition respectively.

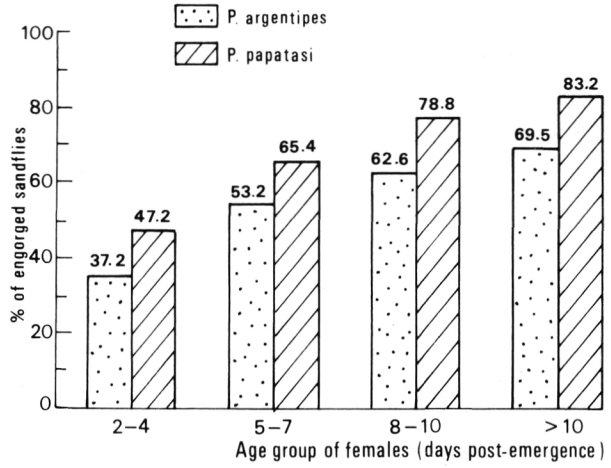

FIG. 2. — Feeding responses of laboratory bred *P. argentipes* and *P. papatasi* of different age groups to host stimuli (man).

TABLE III. — *Feeding activity of* P. argentipes *and* P. papatasi *in laboratory condition.*

Species	*Percentage of engorgement*				
	Albino rats (3-4 hours)	*Guineapigs* (3-4 hours)	*Hamsters* (3-4 hours)	*Human hands* (3-4 hours)	*White mice* (3-4 hours)
P. argentipes	12.2^{a} (8-17)	32.0^{b} (27-38)	52.4^{c} (45-59)	48.8^{d} (39-56)	69.2^{e} (63-74)
P. papatasi	37.6^{A} (31-44)	58.4^{B} (53-64)	66.2^{C} (60-72)	79.6^{D} (71-85)	81.4^{E} (74-87)

Significance difference is observed between a – b ($z = 7.54$), b – c ($z = 6.56$), c – d ($z = 1.14$), c – e ($z = 5.44$), A – B ($z = 6.58$), B – C ($z = 2.54$), C – D ($z = 4.76$), D – E ($z = 0.71$) and between a – A ($\chi^2 = 17.41$), b – B ($\chi^2 = 14.07$), c – C ($\chi^2 = 3.96$), d – D ($\chi^2 = 20.63$), e – E ($\chi^2 = 4.00$)

[$z > 1.64$ and $\chi^2 > 3.841$ are significant at 5 % level and $z > 2.32$ and $\chi^2 > 6.635$ are significant at 1 % level].

TABLE IV. — *Pre-biting period necessary to respond to host stimuli (man).*

Species	*Pre-biting period* (min)	*Percentage of engorgement according to age groups*			
		2-4 (days)	*5-7* (days)	*8-10* (days)	*> 10* (days)
P. argentipes	0-15	30.2^{a} (26-35)	40.2^{b} (34-45)	48.6^{c} (42-53)	59.2^{d} (54-65)
	15-30	27.2 (23-32)	26.4 (23-30)	25.2 (20-31)	26.2 (23-29)
	30-60	23.4 (19-27)	21.2 (17-25)	19.2 (14-24)	11.0 (7-15)
	> 60	19.2 (15-23)	12.2 (9-16)	7.0 (3-10)	3.6 (1-5)
P. papatasi	0-15	37.4^{A} (30-45)	47.4^{B} (42-53)	53.4^{C} (48-57)	68.2^{D} (64-73)
	15-30	28.6 (23-33)	27.2 (23-32)	23.2 (17-29)	21.4 (17-25)
	30-60	22.2 (18-27)	18.2 (13-22)	17.4 (12-23)	8.6 (5-11)
	> 60	11.8 (5-16)	7.2 (4-11)	6.0 (3-10)	1.8 (1-4)

Significance difference is observed between a – b ($z = 2.97$), b – c ($z = 2.67$), c – d ($z = 3.36$), A – B ($z = 3.19$), B – C ($z = 1.89$), C – D ($z = 4.79$), a – A ($z = 2.40$), b – B ($z = 2.29$), c – C ($z = 1.51$) and d – D ($z = 2.96$).

Figures in the parentheses indicate the ranges.

TABLE V. — *Oviposition survival and multiple feeding activity of* Phlebotomus argentipes *and* P. papatasi *in the laboratory.*

Test groups	*Species*	*No. of females exposed*	*1st blood meal*	*1st oviposition*	*1st oviposition survival*	*2nd blood meal*	*2nd oviposition*	*2nd oviposition survival*
1	*P. argentipes*	80	69	51	22	6	3	1
	P. papatasi	65	61	57	42	19	9	5
2	*P. argentipes*	72	58	44	17	5	—	—
	P. papatasi	68	62	53	41	24	11	5
3	*P. argentipes*	95	73	52	26	11	3	2
	P. papatasi	53	49	43	28	10	5	2
4	*P. argentipes*	86	72	49	31	9	3	1
	P. papatasi	56	50	48	32	19	13	7
5	*P. argentipes*	105	86	69	38	14	5	2
	P. papatasi	61	57	42	31	14	8	4
6	*P. argentipes*	79	70	49	19	6	2	—
	P. papatasi	71	66	58	39	18	8	3
7	*P. argentipes*	140	115	86	33	10	6	2
	P. papatasi	140	128	102	63	27	10	5
Total	*P. argentipes*	657	543^{a}	400^{b}	186^{c}	61^{d}	22^{e}	8^{f}
	P. papatasi	514	473^{A}	403^{B}	276^{C}	131^{D}	64^{E}	31^{F}

Significance difference is observed between a – A ($z = 4.69$), b – B ($z = 4.50$), c – C ($z = 7.69$), d – D ($z = 3.21$), e – E ($z = 1.68$) and f – F ($z = 1.69$).

The sandflies usually took about 25 sec to 2 min to mate which usually, but not always, occurred after a blood meal. However, this behaviour does not differ significantly between the species. An adult of *P. argentipes* is capable of taking about 0.22-0.27 mg of blood and *P. papatasi* 0.26-0.30 mg of blood. The blood meal digestion started posteriorly with the follicular development, and eggs were laid by 4-6 days. The egg laying capacity of *P. argentipes* and *P. papatasi* was about 47 ± 10.85 and 39 ± 8.07 respectively.

During the 1st and early 2nd instar stages, larvae of both species usually moved on the surface of the food and from the end of the 2nd instar they started burrowing into the food *i. e.,* positively geotactic. This made the food loose and the presence of larvae can be deduced by the nature of food.The larvae were unable to burrow in overmoistened food caused by excess water in the towel in the tray. The average weight of 4th instar larvae of *P. argentipes* and *P. papatasi* was 0.32 mg and 0.35 mg respectively.

We have seen larvae pupating on the food in the vial but on a few occasions they move up and pupate on the wall of the rearing vial. This was seen particularly in the hot months when a cooling fan was used above the rearing vials. Just before pupation the larvae showed no food in the gut as they came to the surface of the food matter *i. e.,* negatively geotactic.

DISCUSSION

The conducive factors for maintaining a productive laboratory colony of a phlebotomine fly are easy engorgement and low mortality rate (Killick-Kendrick, 1978). These favourable conditions are dependent on various factors, particularly the temperature. The mortality rate of bloodfed flies and the immature stages of *P. papatasi* at a particular temperature *(Table I)* is less than that of *P. argentipes* (Ghosh and Bhattacharya, 1989). A statistical analysis of the results *(Tables I and II)* clearly indicates the advisability of altering the temperature during different phases of rearing of *P. papatasi.*

Oviposition at 29°-30° C gives the best result for *P. papatasi.* Our results are similar to those of Eldridge *et al.* (1963) who observed the egg laying capacity of *P. papatasi* as 40.39 ± 16.19 eggs, although Waterston (1922) obtained slightly higher numbers of eggs (40-50) from this species. With *P. argentipes,* our results coroborate those of Eldridge *et al.* (1963) and Smith (1959) who recorded the egg laying capacity of this species as 47.07 ± 16.75 and 45 respectively. Our results are different from those of Pandya (1980) who reported no difference in the egg laying capacity of the two species which he recorded as 43 ± 4.4 eggs.

An increase in the temperature of the rearing chamber from 20° C to 31° C resulted in better development of the larvae. For this the eggs and pupae were maintained at about 31° C which facilitated the quicker hatching of the larvae and emergence of the adults. Similar effects were also observed in *P. argentipes* (Ghosh and Bhattacharya, 1989). The burrowing habit of the larvae in the food matter, positively geotactic, in 3rd and 4th instars of both species, as shown in this study, gives an idea of their possible existence in natural breeding places, although the larvae of

P. perniciosus did not behave in the same way (Maroli *et al.*, 1987).

In our study, the generation time of *P. papatasi* was longer than that of *P. argentipes*. This observation agrees with that of Eldridge *et al.* (1963) but not with that of Pandya (1980). Eldridge *et al.* (1963) found the generation times to be 35 days for *P. argentipes* and 45 days for *P. papatasi* whereas Pandya (1980) gave figures of 39-45 days for both.

Improved longevity of the adults was observed at 24°-26° C although *P. argentipes* can thrive well at 20°-31° C and *P. papatasi* at 19°-33° C and the longevity is reduced due to the higher temperature *(Tables I and II)*.

Phlebotomus papatasi was a more aggressive feeder than *P. argentipes*. This was shown by the fact that *P. argentipes* took longer than *P. papatasi* for an equal number of engorgement *(Table IV)*. This is in contrast to the observations of Eldridge *et al.* (1963) who pointed out that a longer exposure was required to feed *P. papatasi* than *P. argentipes*. The highest engorgement rate for both species was found with mice when exposed for 3-4 hours although the engorgement rate of *P. papatasi* was higher than *P. argentipes* on every host animal *(Table III)*. The difference in engorgement rate between the two species on human is highly significant. *Phlebotomus papatasi* preferred human to hamster but *P. argentipes* showed no significant difference in choice *(Table III)*. This supports the observations of Dhanda and Gill (1982) and Mukhopadhyay and Chakravarty (1987) that *P. papatasi* is mainly anthropophilic but *P. argentipes* is largely zoophilic. Significant differences in engorgement rates of *P. papatasi* were observed from 24°-26° C to 32°-33° C with highest proportions fed at 27 ± 0.5° C. In contrast, *P. argentipes* showed the highest feeding activity at 25°-26° C (Ghosh and Bhattacharya, 1989).

It appears that some stages of *P. papatasi* favour a slight higher temperature (about 1°-1.5° C) than those of *P. argentipes* and can withstand a slightly wider range of temperature. This observation is also in accordance with the distribution of *P. papatasi* from the temperate zone of Rajasthan to hot places in Bihar State.

The pain due to the bite of 6-9 days old *P. papatasi* was more severe than that of *P. argentipes* of the same age. As the age of the vector advanced the bites became more painful. The reaction persisted longer in *P. papatasi* bite with an inflammed papular area. A papular rash « harara » following *P. papatasi* bite was reported by Theodor (1935) and Adler and Theodor (1957).

As far as multiple feeding activity and oviposition survival are concerned as important factors for vector competence, *P. papatasi* appears to be more efficient than *P. argentipes*. This laboratory observation supports the findings of Mukhopadhyay and Chakravarty (1987) and Dhanda and Gill (1982) who concluded that the frequency of multiple feeding in *P. papatasi* was higher than that of *P. argentipes* in nature. However, the multiple feeding activity and oviposition survival rate of *P. argentipes* was lower than that of *P. perniciosus* and *Lu. longipalpis* as observed by Maroli *et al.* (1987) and Buescher *et al.* (1984) respectively but *P. papatasi* showed higher survival rate than the other two species.

In the light of adult longevity, feeding activity on laboratory animals, the time required to respond to host stimuli, multiple feeding activity and oviposition survival, *P. papatasi* is regarded as a more robust and opportunistic feeder than *P. argentipes*. *Phlebotomus papatasi* is, therefore, preferable to *P. argentipes* for laboratory studies.

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