Rhythmicity in oviposition pattern in light and darkness in four Indian species of *Drosophila*

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Experiments were conducted to test the effect of illumination conditions on oviposition patterns of ***Drosophila ananassae, D. bipectinata, D. malerkotliana*** *and* ***D. biarmipes****. Illumination conditions were: (i) 12:12 light and dark cycle (LD), (ii) 24 h constant light condition (LL) and (iii) 24 h constant dark condition (DD). Females of all the four species laid more eggs under light condition as compared to the dark condition. There is consistency in the results under all the three experimental conditions for all the four species. These findings suggest that light condition itself is favourable to oviposition in these Indian* ***Drosophila*** *species.*

Key terms: *Indian Drosophila species, oviposition pattern, light and darkness, rhythmicity.*

INTRODUCTION

Oviposition is an important aspect of non-sexual behaviour of adult female *Drosophila*. According to Carson (1971), many *Drosophila* species are selective in their choice of ovipositional substrate, whereas most species are opportunistic generalists in their feeding behaviour. Oviposition behaviour plays a central role in the evolutionary ecology of *Drosophila*, because it may affect preadult viability (Jaenike, 1982; Mueller, 1985).

Since *Drosophila* larvae have a low mobility, their survival depends largely on the choice of oviposition sites by their female parents. Thus, choice of oviposition sites seems to be closely related to fitness. Oviposition site preferences of a variety of *Drosophila* species have been reported, and there are intra- and interspecies variations to oviposition site preference (Moore, 1952; Pyle, 1976; Richmond & Gerking, 1979; Takamura & Fuyama, 1980; Takamura, 1984). Choice for oviposition site in *D. melanogaster* and other species is influenced by medium condition, humidity, density of females, previous female presence on the oviposition site, previous presence of eggs on the oviposition sites, temperature, light, ethanol and other chemicals (Mainardi, 1968; del Solar & Palomino, 1970; David, 1970; Barker, 1973; Markow, 1975; Fogleman, 1979; Takamura, 1980; Jaenike, 1982; Wogaman & Seiger, 1983). Evidence for genetic control of oviposition behaviour has also been reported in *D. melanogaster* and *D. pseudoobscura* (del Solar, 1968; Takamura & Fuyama, 1980; Seiger & Sanner, 1983; Albornoz & Dominguez, 1987; Gonzalez, 1989; Kamping & Van Delden, 1990; Van Delden & Kamping, 1990; Ruiz-Dubreuil & del Solar, 1991; Ruiz-Dubreuil *et al*, 1992).

Effect of illumination conditions on oviposition behaviour has been investigated in various *Drosophila* species (Gruwez *et al*, 1971; Kambysellis & Wheeler, 1972; Allemand, 1976, 1982; Ohnishi, 1977; Kawanishi & Watanabe, 1978; Wogaman & Seiger, 1983; Seiger & Sanner, 1983). In *D. melanogaster*, egg production is higher in dark phase than in light phase under light-dark cycle (Gruwez *et al*, 1971;

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Allemand, 1976), which is due to higher ovarian activity during illuminated period. Contrary to this, Ohnishi (1977) found that females of *D. melanogaster*, *D. lutescens* and *D. virilis* laid more eggs in light than in dark. A similar result was reported by Kambysellis and Wheeler (1972) in *D. virilis*. Natural populations of *Drosophila ananassae*, *Drosophila bipectinata*, *Drosophila malerkotliana* and *Drosophila biarmipes*, collected in India may differ with respect to a variety of behavioural traits (Singh & Chatterjee, 1985, 1986, 1987, 1988a,b, 1989; Singh & Pandey, 1991, 1993a, b, 1994; Srivastava & Singh, 1993a, b). We have tested the effect of light and dark on oviposition pattern of these four Indian species of *Drosophila* and the results are here reported.

MATERIALS AND METHODS

Experiments were carried out to test the effect of light and darkness on oviposition behaviour in four species of *Drosophila*. These species are *D. ananassae* (strain B-84, geographic origin - Bhubaneswar, Orissa, established in 1984), *D. bipectinata* (BHU stock, geographic origin - Banaras Hindu University, established in 1987), *D. malerkotliana* (Baripada strain, geographic origin-Baripada, Orissa, established in 1987) and *D. biarmipes* (Ng strain, geographic origin-Nagpur, Maharashtra, established in 1990). All these stocks are being maintained on simple culture medium under 12:12 LD cycle and have spent varying number of generations in the laboratory. In all the experiments, simple culture medium containing agar agar, dried yeast, maize powder, brown sugar, nipagin, propionic acid and plain water was used. In order to facilitate the counting of eggs, a green edible dye was added to the medium and spot of active yeast was placed in the centre of food medium.

Stocks of all the four species were cultured and virgin females and males were collected. Flies were aged for two days. Single female and male were kept in a food vial for two days for mating. Then the flies were transferred to a food vial (8 cm length x 3 cm diam.) without etherization.

Experimental conditions: The oviposition pattern in four species was examined under the following three different conditions: *i*- alternate light and dark (12:12 **LD** cycle), consisting of 12 h light (6 a.m. to 6 p.m.) and 12 h dark (6 p.m. to 6 a.m.) phases; *ii*- constant light condition (**LL**); *iii*- constant dark condition (**DD**).

Under 12:12 LD cycle, flies were transferred to fresh food vials every 12 h and number of eggs in each original vial was counted. Under 24 h continuous light (LL) and 24 h continuous dark (DD) conditions, flies were transferred to fresh food vials every 24 h and number of eggs in each original vial was counted (6 a.m.). In all the experiments, oviposition pattern was studied for four consecutive days. In each species, oviposition of 40 females (♀) was examined in each experimental condition. The data of those vials in which females died during the course of observations were rejected. Therefore, the number of females may vary for each species. In light, the experiments were conducted under laboratory light conditions (about 1000 lux). In dark, the vials were covered with black paper and flies were transferred in dark. These vials were kept in light tight ventilated chamber. All the experiments were conducted at 24 ± 1° C with 60-80% RH. Comparisons for mean numbers of eggs/♀/12 h and 24 h light and dark conditions were done by Student's *t*-tests.

RESULTS

Mean number of eggs/♀/12 h in light and dark conditions (12L:12D cycle) in four species is given in Table I and depicted in Figure 1. In these experiments, eggs were counted every 12 h for four days. In all the four species, females laid more eggs in the light phase of the light-dark cycle. The differences in mean number of eggs between light and dark phases are statistically highly significant in all cases (Table II). The egg production is higher in light compared to dark and thus there is rhythmicity for oviposition in these four Indian species of *Drosophila*.

Experiments were also conducted to study oviposition in all the four species under constant light (LL) and constant dark (DD) for four days and eggs were counted every

Table I

Mean number of eggs/♀/12 h in light and dark conditions (12L:12D cycle) in four species of *Drosophila*

Species	N° of ♀ tested	N° of days	Total N° of eggs counted		N° of eggs /♀/ 12 h Means ± SE	
			Light	Dark	Light	Dark
D. ananassae	38	4	1698	84	11.17 ± 1.21	0.55 ± 0.06
D. bipectinata	34	4	1510	308	11.10 ± 1.08	2.26 ± 0.26
D. malerkotliana	39	4	1446	188	9.26 ± 0.19	1.20 ± 0.28
D. biarmipes	40	4	738	390	4.61 ± 0.36	2.43 ± 0.34

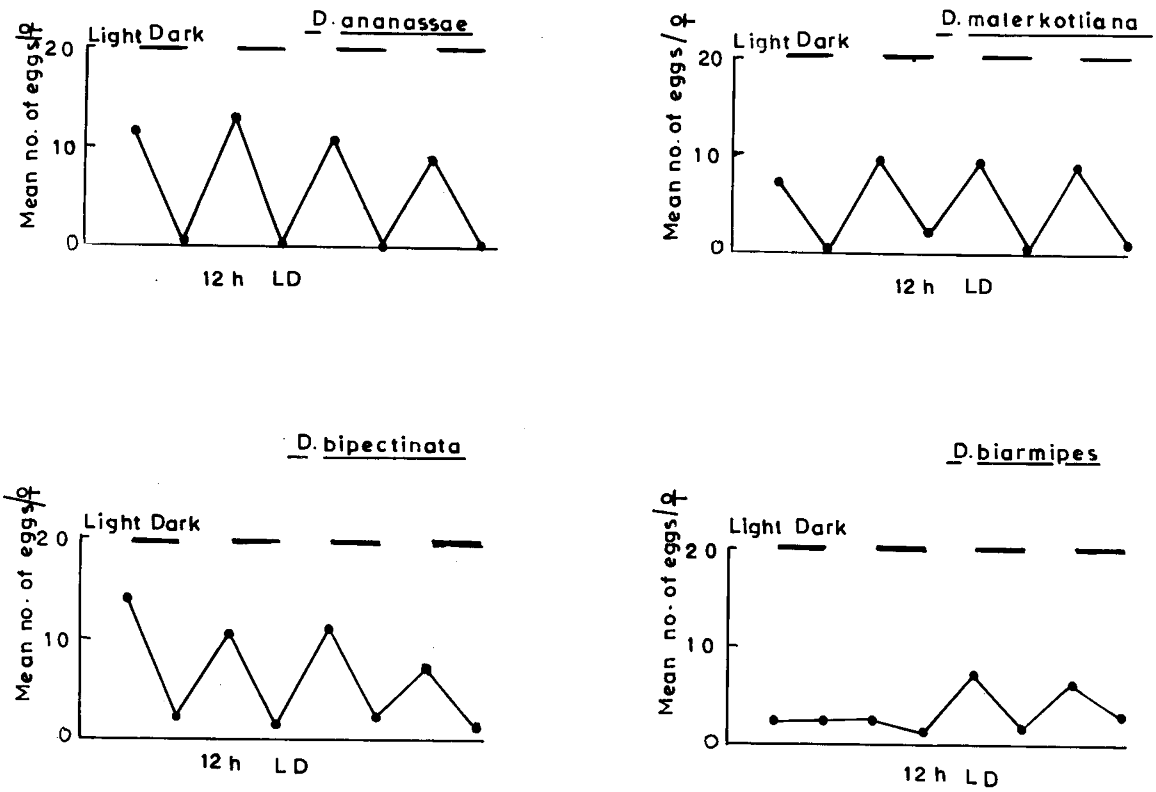

Fig 1. Mean number of eggs/♀/12 h in light and dark conditions (12L:12D cycle) in four species of *Drosophila*.

24 h. Table III presents data on mean number of eggs/♀/24 h in four species under constant light and constant dark conditions. Mean number of eggs in all the four species is higher in constant light than in constant dark. The difference in the mean number of eggs between constant light (LL) and constant dark (DD) is statistically significant (see Table IV). Thus, females of all the four species oviposit more eggs under light conditions than under darkness.

DISCUSSION

Three species *D. ananassae*, *D. bipectinata* and *D. malerkotliana* belong to the *ananassae* subgroup of the *melanogaster* species group, whereas *D. biarmipes* belongs to the *suzukii* subgroup of the *melanogaster* species group. *D. ananassae* is a cosmopolitan and domestic species, while *D. bipectinata* and *D. malerkotliana* are semi-wild species which coexist with each other and also with

Table II

Comparison of mean number of eggs/♀/12 h between light and dark conditions (12L:12D cycle)

Species	Light Mean ± SE	*vs*	Dark Mean ± SE	*t*	df	p <
D. ananassae	11.17 ± 1.21		0.55 ± 0.06	8.85	37	0.001*
D. bipectinata	11.10 ± 1.08		2.26 ± 0.26	7.89	33	0.001*
D. malerkotliana	9.26 ± 0.19		1.20 ± 0.28	11.04	38	0.001*
D. biarmipes	4.61 ± 0.36		2.43 ± 0.34	5.73	39	0.001*

* Significantly different.

Table III

Mean number of eggs/♀/24 h in continuous light and continuous dark conditions in four species of *Drosophila*

Species	N° of ♀ tested		N° of days	Total N° of eggs counted		N° of eggs /♀/ 12 h Means ± SE	
	Light	Dark		Light	Dark	Light	Dark
D. ananassae	33	30	4	2134	523	16.16 ± 1.26	4.35 ± 0.61
D. bipectinata	38	40	4	1084	559	7.13 ± 0.53	3.49 ± 0.37
D. malerkotliana	39	31	4	1584	169	10.15 ± 0.82	1.36 ± 0.03
D. biarmipes	39	36	4	938	337	6.00 ± 0.45	2.34 ± 0.44

Table IV

Comparison of mean number of eggs/♀/24 h between continuous light and continuous dark conditions

Species	Light Mean ± SE	*vs*	Dark Mean ± SE	*t*	df	p <
D. ananassae	16.16 ± 1.26		4.35 ± 0.61	8.11	61	0.001*
D. bipectinata	7.13 ± 0.53		3.49 ± 0.37	5.68	76	0.001*
D. malerkotliana	10.15 ± 0.82		1.36 ± 0.03	10.10	68	0.001*
D. biarmipes	6.00 ± 0.45		2.34 ± 0.44	5.43	73	0.001*

* Significantly different.

D. biarmipes, which is less frequent than the other three species. Females of all the four species show tendency to insert eggs into the substrate and also prefer the peripheral area of the culture medium to oviposit eggs (Srivastava & Singh, 1993 a, b). There are also intra- and interspecies variations in oviposition site preferences. Evidence for genetic control of mating behaviour and pupation height in *D. ananassae* has been presented (Singh & Chatterjee, 1986; 1988a,b; Chatterjee & Singh, 1987, 1988; Singh & Pandey, 1993a, b). Except for *D. bipectinata*, all the three species show higher pupation height under dark condition (Pandey & Singh, 1993).

It is evident from the results here presented that females of all the four species lay more eggs in light than in dark when tested under 12L: 12D cycle (Fig 1). Thus, there is rhythmicity in the oviposition behaviour of these four species. Further, all the four species show higher fecundity under constant light as compared to constant dark. These findings suggest that light condition is favourable to oviposition in all the four

Indian species, although these species differ in other aspects of their ecology: *D. ananassae* is a cosmopolitan and domestic species, whereas *D. bipectinata*, *D. malerkotliana* and *D. biarmipes* are semi-wild and have restricted geographical distribution. *D. bipectinata* and *D. malerkotliana* are closely related sympatric species, but still they do not show photobehavioural difference.

Photobehavioural differences have been found among sympatric populations of sibling species *D. pseudoobscura* and *D. persimilis* (Wogaman & Seiger, 1983). The different photopreference of the sibling species *D. simulans* (photopositive) and *D. melanogaster* (photo-neutral) could be a possible cause of co-existence of these two species in nature (Kawanishi & Watanabe, 1978). It is likely that photobehaviour in relation to oviposition site is adaptive, and niche separation is most understandable in an evolutionary context (Wogaman & Seiger, 1983).

Our results contrast with those reported for *D. melanogaster*, which show maximum egg-laying during the dark phase when maintained under light-dark cycle (Gruwez *et al*, 1971; Allemand, 1976). It is thought that the ovariole activity could be higher during the illuminated period than during darkness (Gruwez *et al*, 1971). On the other hand, larger egg production was found under light phase than under dark phase in *D. melanogaster*, *D. lutescens* and *D. virilis* (Kambysellis & Wheeler, 1972; Ohnishi, 1977). This can be explained by one of the following two mechanisms: *i*- more eggs may be produced by the stimulus of dark to light alteration, so that the egg production in the light phase might be larger than the darker phase; *ii*- the light condition itself may be favourable for egg production (Ohnishi, 1977). The larger egg production during the light phase in the Indian species of *Drosophila* employed during the present study also suggests that light condition itself is favourable for oviposition, as suggested by Ohnishi (1977). However, larger egg production under constant light than under constant dark observed during the present study could indicate the involvement of steady stimuli rather than dark to light alteration for larger egg production. Other designs are needed to study LD shorter or longer periods.

ACKNOWLEDGEMENTS

We thank two anonymous reviewers for their comments on the original draft of this manuscript.

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