Latitudinal variation in eclosion rhythm among strains of *Drosophila ananassae*

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Received 23 July 1998; revised 11 January 1999

Eclosion rhythm parameters of *D. ananassae* strains originating between 8°-34° N were highly variable and latitude dependent. In the field under naturally fluctuating light intensity, temperature and R.H., the amplitude of the rhythm was high and the eclosion gate was narrow; however, under the naturally fluctuating light intensity but at constant temperature and R.H., the amplitude of the rhythm was lowered and the width of eclosion gate was widened. The eclosion rhythm entrained to light-dark (LD) cycles ranging from LD 6:18 to LD 18:6, the width of the eclosion gate was decreased and increased in the short and long photoperiods respectively. Among the strains, both the phase angle difference (Ψ, the time from lights-off in a 24 hr LD cycle to the eclosion median) and the period of free-running rhythm (τ) in constant darkness varied by about 3 hr and the amplitude of the rhythmicity (Amp) by about 10%. Lower latitude was correlated with late Ψ (r = -0.69), long τ (r = 0.88) and high Amp value (r = -0.95).

Genetic variability in the properties of the circadian clock of an organism can be studied by various ways. For example, artificial selection was used to establish "early" and "late" strains in the eclosion rhythm of *Drosophila pseudoobscura* and in *D. melanogaster*. Artificial selection for developmental time in the melon fly, *Bactrocera cucurbitae* had also affected its free running period of locomotor activity. Mutagenic agents were used to induce mutation at the *per* locus in *D. melanogaster*; similarly spontaneous mutations were studied in golden hamsters and mice or in the mosquito *Aedes krombein*. Genetic variability can also be studied by screening the wild populations originating from different latitudes. For example, 12 strains of *D. subobscura* originating from 28°-63° N were found to be variable in eclosion rhythm parameters. This last approach has potential evolutionary and adaptive significance. However such reports on *Drosophila* are published mostly on the strains from the temperate regions. Such strains are evolved under entirely different environmental conditions than those from the tropical regions.

The interest to investigate the circadian rhythm of eclosion in the tropical fruit-fly *Drosophila ananassae* was initiated after carrying out the preliminary field experiments on the KK-15 strain from Kanyakumari (8.1°N). The results revealed that during entrainment to natural light-dark (LD) cycles when environmental variables like temperature, light intensity, % relative humidity (R.H.) etc. were included, the amplitude of the eclosion rhythmicity was very high, i.e., more than 90% of the total flies eclosed during one hour eclosion median before sunrise. Then we started to search for the latitudinal variation in the diel eclosion rhythm in the natural populations of this species in India. The field experiments proved to be rewarding which were then repeated in the laboratory under controlled environmental conditions to investigate the role of light when temperature and R.H. were maintained constant. In the present article, the genetic variability in the circadian eclosion rhythm in the latitudinal strains of *D. ananassae* originating between 8°-34° N in India has been investigated.

**Materials and Methods**

*D. ananassae* is a cosmopolitan and domestic species in India. The 27 strains as shown in Table 1 were originated from single females captured at 9 latitudes. This table also gives the latitude, longitude, altitude and the year of capture of the strains. Details of rearing method, composition of the culture medium and technique of harvesting the pupae have been described. The eclosion rhythm was monitored with infrared sensors as used for *D. pseudoobscura*.

The data for eclosion are plotted as percentages of the daily eclosions per hour obtained by totalling the number of eclosion events over a 24 hr cycle.
The eclosion rhythm in all strains during entrainment to LD cycles and free-runs in continuous darkness (DD). The phase angle difference (Δφ) of the eclosion rhythm during 5-7 days of entrainment to LD 12:12 was defined as the time from lights-off to the eclosion median. During entrainment to natural LD cycles in the field, the width of the eclosion gate was determined. It was defined as the total number of hours during which the eclosion events lasted (Figs 1 and 2).

Free-runs of the rhythm were initiated by transferring the pupae from continuous light (LL) at about 100 lux to DD. The period of the free-running rhythm (τ) was determined by fitting a least square regression line to the eclosion medians on the third day onwards in DD when the initial transient cycles subsided. Amplitude of the rhythmicity (Amp) in the free-running state was estimated as the mean of the three highest hourly percentages in each daily eclosion peak over the peaks 3-6. The value of Amp could lie between 4.17% (arhythmity) to 33.3% (all three peaks within 3 h each).

Three strains representing widely separated latitudes were selected for thorough analysis. They were as follows: KK-15 from Kanyakumari; AC-11 from Ahmednagar College, Ahmednagar and DS-23 from Drass. Two sets of field experiments were performed on these strains at the origin of their latitude. The experimental site was located in house gardens where these flies breed in nature. In the first set of experiments, the flies were reared and bred in open space in the garden so that they were exposed to all rhythmically changing environmental variables like temperature, light intensity, R.H., etc. But in the second set of experiments, the flies were reared and bred at constant temperature and R.H. as follows. The culture bottles were kept in clear glass chambers (2×2×2 m) supported by aluminium frames. The temperature was maintained at 25°±0.5°C and the R.H. about 80% by using air-conditioning machine and humidifier respectively. Four replicate recordings from each strain were simultaneously studied for 5-7 days till all flies eclosed. The light intensity was measured at 10 min intervals, whereas the temperature and R.H. were recorded continuously. They are shown only for the period of 2 hr prior to and 2 hr after the sunrise (SR) (Figs. 1 and 2, the right panels).

The strains were maintained as mass culture on corn flour medium in LL of about 100 lux and at 25±0.5°C and 80% R.H. after their arrival in our laboratory. Entrainment to LD 12:12 was studied in all 27 strains. The 3 representative strains were additionally studied in five LD cycles; they were as follows: LD 6:18; LD 9:15; LD 11:13; LD 15:9 and LD 18:6. The light intensity was about 100 lux during the photophase and absolute darkness during the scotophase in all LD cycles. The maintenance in darkness was carried out by using very dim diffused red light (2.1×10^12 photons/cm^2/s) of the wavelength > 660 nm. The results of entrainment and free-runs are based on 4 replicate recordings for each strain.

### Results

Three parameters of eclosion rhythm of D. ananassae were analysed in 27 strains from 9 latitudes as presented in Table 2. The entrainment in the field when natural daily fluctuations in temperature, light and R.H. were included is shown for the 3 representative strains in Fig. 1. The eclosion medians in all 3 strains occurred during the dawn
twilight just 1 hr before the sunrise (Fig. 1, left panels). However, the latitudinal differences were evident in the eclosion patterns. In KK-15 strain, the width of the eclosion gate was 3.1 hr (SD±0.2 h, N=20, total number of eclosion gates) and about 90% flies eclosed during 1 hr median; in AC-II strain, the gate-width was 4.2 hr (SD±0.4 h, N=20) and about 70% flies eclosed during the median hour; in DS-23 strain the gate-width was 9.2 hr (SD±1.1 h, N=21) and only some 45% flies eclosed during the median hour. There was about 1,000 times steep increment in the light intensity during this one hour period at these three latitudes. The increment in light intensity was accompanied by slight increment in temperature and reduction in R.H. (Fig. 1, right panels). Fig. 1 shows the single repeats of eclosion records on June 6, 1996 for KK-15 strain at Kanyakumari, on June 30, 1996 for AC-11 strain at Ahmednagar and on July 14, 1996 for DS-23 strain at Dras.

The entrainment in the field at constant temperature and R.H. is shown in Fig. 2. The width of the eclosion gate was increased in each strain. It was 5.2 hr (SD±0.4 h, N=20) in KK-15 strain; 7.2 hr (SD±0.3 h, N=22) in AC-11 strain, and 11.2 hr (SD±1.1 h, N=21) in DS-23 strain. This has lowered the magnitude of the eclosion median in each strain. Thus, some 70% flies of the KK-15 strain, about 55% flies of the AC-11 strain and 38% flies of the DS-23 strain eclosed during the eclosion median just 1 hr before the sunrise. In all strains the eclosion continued 2-6 hr well after the sunrise. The steep increase in light intensity is shown in the right panels of the Fig. 2. It shows the single repeats of eclosion records on June 7, 1996 for KK-15 strain at Kanyakumari, on June 29, 1996 for AC-11 strain at Ahmednagar and on July 15, 1996 for DS-23 strain at Dras.

![Table 2—Four parameters of the eclosion rhythm in 27 strains of D. ananassae: the phase angle difference (Ψ, h) during entrainment to LD 12:12, the free-running period in DD (τ, h) and % amplitude of free-running rhythmicity (Amp) in DD.](image)

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<th>τ, h</th>
<th>±SD</th>
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Fig. 1—Diel eclosion rhythm showing single repeats for the 3 strains of D. ananassae recorded in the field at the origin of their latitude when natural daily fluctuations in light intensity, temperature, and % R.H. were included. The left panels show eclosion records of KK-15 strain at Kanyakumari (8.1°N), AC-11 strain at Ahmednagar (18.9°N) and DS-23 strain at Drass (34.3°N). The right panels show changes in light intensity, temperature and R.H. 2 hr prior to and 2 hr after the sunrise (SR). The arrows in the right panels indicate the onset of astronomical twilight at each latitude.
The entrainment to LD 12:12 cycles in the laboratory was studied for 5 days in all 27 strains. The \( \Psi \) varied by about 3 hr among the strains (Table 2). The results shown in Table 2 are based on the pooled data for six replicate recordings per strain. Low latitude was correlated with late \( \Psi (r = -0.69, P < 0.0001) \). The 3 representative strains were additionally studied in five LD cycles ranging from LD 6:18 to LD 18:6 and the results of such single repeats are presented in Fig. 3. The entrainment pattern among the strains remained principally the same as in LD 12:12. In all LD cycles the \( \Psi \) was always earlier in the DS-23 strain as compared to the \( \Psi \) in KK-15 or AC-11 strain. In very short photoperiod (LD 6:18) the width of the gate was reduced and eclosion medians shifted to the lights-on in all strains (Fig. 3, top panel). Whereas in very long photoperiod (LD 18:6), the width of the gate was increased and the eclosion medians shifted into the photophase in all strains (Fig. 3, bottom panel). The results of entrainment to 5 LD cycles in the 3 representative strain are based on 4 replicates of each strain.

The free-runs initiated by LL/DD transfers are shown in Fig. 4 for the 3 representative strains. The steady-state free-runs were established usually after 1-2 transient cycles in all strains. Among 27 strains, the \( \tau \) varied by about 2.5 hr, and the Amp by about 10% (Table 2). These results are based on the pooled data for six replicate recordings per strain. Low latitude was correlated with long \( \tau (r = -0.88, P < 0.0001) \) and high Amp \( (r = -0.95, P < 0.0001) \).

**Discussion**

In nature the timing of pupal eclosion should be in

![Graph](https://via.placeholder.com/150)

Fig. 3—Eclosion rhythms showing single repeats for the 3 representative strains of *D. annassae* in LD cycles ranging from LD 6:18 to LD 18:6 at 25°C and 80% R.H. in the laboratory. The eclosion medians are always early in DS-23 strain in all LD cycles.

![Graph](https://via.placeholder.com/150)

Fig. 4—Free-running rhythms of eclosion showing single repeats in the 3 strains of *D. annassae* initiated by LL/DD transfer at the beginning of day 1. Note that \( \tau \) in DS-23 = \( \tau \) in AC-11 = \( \Psi \) in KK-15.
phase with the environmental cycles of light, temperature and R.H. It appears to be under the control of natural selection. In most species of *Drosophila* so far reported, the eclosion has well-defined rhythm with peak close to dawn. At this hour, the temperature is moderate and the R.H. is highest which help the newly eclosed pharates to expand their wings without getting desiccated. The tropical fruit-fly *D. ananassae* having a wide latitudinal distribution in India is expected to evolve with proper phase of eclosion to the dawn of the latitude of its origin. However, the three strains of *D. ananassae* studied in the field eclosed during dawn twilight with the eclosion medians occurring 1 hour prior to the sunrise when the daily natural fluctuations in temperature and R.H. were included (Fig. 1, left panels) or excluded (Fig. 2, left panels).

Thus the specific stable segment of the dawn twilight during pre-sunrise hour at these latitudes seems to be the primary photic zeitgeber for eclosion in *D. ananassae*. During this time the rate of change in light intensity is greatest. The light intensity increases from $10^2$ lux to $10^3$ lux, i.e., a steep increase of $10^3$ times in less than 1 hr (Figs 1 and 2, right panels). Such changes in light intensity are abrupt in the tropics and remarkably precise in timing from day to day, in spite of fog, cloud cover and other weather conditions. In several animals the twilight period serves as an unequivocal environmental reference point for the onset or end of activity. Even simulated twilight periods also raised the upper limit of entrainment and increased the strength of the LD zeitgeber in the hamsters.

Apart from the changes in light intensity during dawn twilight, the temperature and R.H. also play a significant role in phasing the eclosion median in *D. ananassae*. In fact, the very survival of the southern strains depends on the environmental temperature and R.H. as seen from the results of the third set of field experiments on these strains which will be discussed elsewhere (unpublished observations). The flies of the KK-15, AC-11, EL-03 and NL-02 were maintained and bred in the field at 25°C and 80% R.H. till the moment of eclosion (see Materials and Methods). Then they were subjected to eclose at naturally raised high temperature and reduced R.H. by transferring the pupae from the environmentally controlled glass chambers to the field conditions outside. All pharates died in less than two hours, probably because of desiccation. Thus it appears that the natural selection favours the timing of eclosion peak during pre-dawn hours at these latitudes. This might have contributed to the high amplitude of the rhythmicity and very narrow gate of eclosion under field conditions in KK-15 and AC-11 strains of *D. ananassae*. In another tropical insect *Aedes kroebini*, such a high amplitude of the oviposition rhythm had also been reported. Perhaps this may be a generalised phenomenon among other insects of the hot tropical regions.

The environmental temperature and R.H. seem to determine the width of the eclosion gate in *D. ananassae*. When the temperature and R.H. were kept constant, the eclosion in all strains was stretched well into the day-light period for 3-5 hr (Fig. 2, left panels). These results suggest that if 'permissible' temperature and R.H. are offered, the flies do not stop eclosing abruptly 1 hr after the sunrise as observed in naturally fluctuating temperature and R.H. (Fig. 1). This assumption could further be supported by the results of the laboratory experiments on *D. ananassae* when they were bred at constant temperature 25°C and 80% R.H. in LD 12:12 for 17 generations, there was a general tendency towards widening of the gate and lowering of the amplitude of the eclosion rhythm (unpublished observations). Allemand and David also demonstrated the reduction in the amplitude in the diel oviposition rhythm of the Afrotropical populations of *D. melanogaster* after 100 generations of inbreeding at constant temperature in the laboratory.

The latitudinal differences in $\Psi$ of the eclosion rhythmicity during entrainment to LD 12:12 cycles were observed among 27 strains of *D. ananassae* (Table 2). The most extreme pair of strains, i.e., the KK-15 strain and DS-22 strain differed in their $\Psi$ value by about 3.7 hr. In the 3 representative strains the entrainment was additionally studied in other five LD cycles (Fig. 3). In all LD cycles the early eclosion phase was observed in the northern strain, i.e., DS-23 strain, whereas the late eclosion phase in the southern strain, i.e., KK-15 strain. The lights-on signal of all LD cycles seems to be the phase reference point for the eclosion median in these strains. Moreover, the amplitude of the rhythm appears to depend on the duration of the photoperiod than the duration of the dark period of the LD cycles with $T = 24$ hr. The amplitude is very high in short photoperiod, for example, LD 6:18 (Fig. 3, top panel) and very low in long photoperiod, for example, LD 18:6 (Fig. 3, bottom panel). If the duration of photoperiod is more...
than 18 h per 24 hr, the rhythmicity is abolished and the flies eclosed at all hours in these strains of *D. ananassae* (unpublished observations). However, it is still not convincing if the duration of light is more important or that of the darkness. It would be interesting to perform experiments in which the duration of darkness is kept constant while the duration of light varies and vice versa. Four latitudinal strains (41°N to 65°N) of *D. littoralis* were also studied in 7 LD cycles (T = 24 hr) and it was found that the eclosion medians did not have fixed phase reference point with respect to the lights-on or lights-off, however, the eclosion gate was narrowest in all strains when the eclosion medians were at the lights-on. Latitudinal differences in timing of eclosion phase in other species of *Drosophila* have also been reported. Thus the geographical or latitudinal variation in eclosion parameters among natural populations of *Drosophila* species appears to be universal.

The latitudinal variations in τ and Amp were also observed in all 27 strains of *D. ananassae* (Table 2). The northern strains had short τ, and low Amp value. In general, early eclosion median was correlated with short τ and the late eclosion median with long τ as explained from a model designed for the eclosion rhythm of *D. pseudoobscura* by Pittendrigh. The present results on *D. ananassae* agree with this model. Latitudinal variations in τ were reported in other species of *Drosophila* where northern strains showed shorter τ values. For example, in *D. littoralis* and in *D. subobscura*, the early eclosion phase in the northern strains was correlated with short τ.

Induced mutations in *Drosophila* species also yield such results. For example, the *per* mutation in *D. melanogaster* advanced the Ψ of eclosion and shortened τ of both the eclosion rhythm and the adult locomotor activity rhythm in a similar manner, implying that the mechanisms that controls these two behavioural rhythms had at least one molecular component in common or the same endogenous oscillator controlled these two rhythms. The lark mutation in *D. melanogaster* also advanced the Ψ of eclosion in both LD and temperature cycles, however, it did not affect the τ or Ψ of the adult locomotor activity rhythm. Similarly, the *psi-2* and *psi-3* mutations in *D. melanogaster* had advanced the eclosion median but instead of shortening the τ they had lengthened it. This is contrary to the present results on *D. ananassae*. In all strains of *D. ananassae* the early Ψ of eclosion median was always associated with short τ and higher latitude (Table 2). However, there are few exceptions to this, for example, in *D. auraria*, τ was longer in the northern strains than that of the southern strains. Similarly, the “early” strains of *D. pseudoobscura* had early eclosion median but long τ and “late” strain had late eclosion median but short τ.

**Acknowledgement**

These studies are supported by the grant from DST, New Delhi under the Research Project SP/SO/C14/94 to DSJ. We thank many people (too numerous to mention here) for their help in collecting fly strains from all over India. The authors also thank Mr P Gore for fabricating the eclosion recording units, and B M Chatate, M N Kanojiya, S D Joshi, C S Shinde, R G Gandhe and D M Kamble for technical assistance.

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