Chapter – III

Altitudinal variation in phase response curves for the Himalayan strains of *Drosophila helvetica*
INTRODUCTION

Altitudinal or latitudinal variations in basic parameters of the circadian pacemaker controlling eclosion, oviposition and adult locomotor activity rhythms of the naturally occurring clock phenotypes of the fruit fly, *Drosophila* are regarded as a consequence of natural selection (1, 2, 3, 4). Eclosion rhythm parameters of the Japanese strains of *D. auraria* (5, 6), the European strains of *D. littoralis* (7, 8, 9) and *D. subobscura* (10), and the Sri Lankan and Indian strains of *D. ananassae* (11) were found to be latitude dependent and had functional relationship with the prevailing environmental conditions at the latitude of origin. Similarly, the genetic variability in oviposition rhythm parameters of *D. melanogaster* (12, 13) and *D. ananassae* (3, 4) was ascribed to the latitude of origin. Molecular polymorphism in the period gene of wild caught populations of three *Drosophila* species from Europe, Africa and Australia had physiological significance in the context of temperature tolerance and survival (14, 15, 16, 17, 18, 19). Geographic variations in parameters of the adult locomotor activity rhythm of 72 strains of *D. ananassae* (1) and eleven diverse species of *Drosophila* from the US had adaptive relevance (20). Similarly, altitudinal variation in the oviposition rhythm of *D. buzzatii* (21) as well as in the oviposition and eclosion rhythms of *D. ananassae* (2, 3, 4, 22, 23, 24) were found to have ecological significance. Thus, there are several reports detailing the altitudinal variability in eclosion and oviposition rhythms of *Drosophila*, however, there are no published reports on the altitudinal variation in adult locomotor activity of *Drosophila* except for the recent two studies from the authors’ laboratory on the Himalayan strains of *D. helvetica* (25, 26).
The present study is a continuation of ongoing investigations in our laboratory on the effects of altitude of origin on circadian parameters of adult locomotor activity rhythm of the Himalayan strains of *D. helvetica*. The high altitude Himalayan strain of *D. helvetica* was characterized by unimodal activity pattern with a delayed peak during entrainment, and long period of free-running rhythm ($\tau$) in constant darkness (DD), while the low altitude Himalayan strain of the same species had bimodal activity pattern with early morning peak and short $\tau$ in DD (25). Recently, I have also demonstrated that the delayed activity bout of the haH strain could be split into two discrete components, the morning one with $\tau < 24$ h and the evening one with $\tau > 24$ h (26). Furthermore, the haH strain had diminished photic sensitivity like high altitude strains of *D. ananassae* (3, 4, 22, 23, 24). Thus, the altitude of origin of the haH strain affected two important properties of the circadian rhythmicity, the entrainment and persistent free-running rhythmicity in DD. Aim of the present study was to examine whether the altitude of origin of the haH strain also affected the third important property of circadian rhythmicity, i.e., phase shifting of the rhythm free-running in DD. This was achieved by determining the light pulse induced phase response curve (PRC) for the locomotor activity rhythm of the haH strain and compared it with that of the laH strain. The time course and wave form of the PRC for the haH strain were quite different from those of the laH strain.
MATERIALS AND METHODS

The following studied conforms to the ethical standards of research on chronobiology (27). Laboratory populations of the high altitude Himalayan (haH) strain of *D. helvetica* (Burla, 1948) originating from Hemkund-Sahib (4,121 m above sea level, 30.81°N, 79.81°E) and the low altitude Himalayan (laH) strain originating from Birahi (1,132 m a.s.l., 30.62°N, 79.65°E) were derived from 11 and 19 wild-caught gravid females in the field, respectively, in August 2006. Flies were maintained on standard cornmeal medium at 20 ± 0.5°C and ~60% relative humidity (r. h.) under cycles of 12 h of white light at 100 lux and 12 h of complete darkness (LD 12:12). Males of these strains were used for determining the light pulse induced phase response curves (PRCs) for the circadian rhythm of adult locomotor activity. Activity rhythm of individual males (age, 3 days post-eclosion) placed in activity monitors was recorded by the computerized method described elsewhere in details (1, 25). Each activity monitor was housed in a light-tight ventilated wooden box illuminated by fluorescent lamp (Bajaj Ecolux CFL 2W-240V) wired to a digital time switch. Flies were entrained by LD 12:12 cycles prior to releasing in DD and then allowed to free-run for 10-12 days. Onset of activity was considered as the phase reference point of the rhythm as it was more precise and predictable phase point than the mid-point or end of activity. The phase of activity onset (ψ₀) was defined as the time from the lights-on of LD cycle to the time of activity onset as given by an eye-fitted line to ~10 successive activity onsets. Period of free-running rhythm (τ) in DD was computed by fitting a least square regression line to 11 successive activity onsets.
Phases of free-running rhythm were expressed in hours circadian time (Ct) where 1 hour Ct equals $\tau /24$. The phase Ct 0 defines the lights-on. The $\Psi_o$ of each strain in LD 12:12 cycles was assumed to define the same phase of the oscillator in free-running condition in DD, therefore Ct 0 becomes 4.2 h Ct before and 1.1 h Ct after the onset of activity of the $haH$ and $laH$ strains, respectively. Light pulses of 1 h duration at 100 lux were administered to 12 phases of free-running rhythm in DD. Each fly received only one pulse during its free-run as $\tau$ changed due to ‘after-effects’ of light pulses following phase shift. The steady-state phase shift was obtained on the post-pulse day one which was denoted by the difference in Ct hours between the projected timing of activity onset as given by the pre-pulse regression line and the actual timing of activity onset as given by the post-pulse regression line. The PRC for each strain was determined by plotting steady-state phase shifts (mean $\pm$ SD, $N = 5$) as a function of the circadian phase at which light pulses were administered. Scales of the abscissa and ordinate were expressed in hours Ct. The ratio for the advance region to delay region (A/D) in each PRC and the ‘dead zone’, i.e., the phases at which phase shifts could not be evoked with light pulses used in the present experiments, were determined in each PRC.

RESULTS

Both strains of $D. helvetica$ were entrained by LD 12:12 cycles and $\Psi_o$ of the $haH$ strain was $4.1 \pm 0.5$ h ($N = 21$ for this and subsequent values) and $\Psi_o$ for the $laH$ strain was $1.1 \pm 0.3$ h. Figure 3.1 shows the double-plotted activity records of the representative male of
the *haH* strain (A) and *laH* strain (B) entrained by LD 12:12 cycles for the first 7-8 days. Free-running rhythmicity in both strains was initiated by releasing them from LD 12:12 cycles to DD. $\tau$ of the *haH* and *laH* strains were 26.3 $\pm$ 0.4 h and 21.2 $\pm$ 0.3 h, respectively. Light pulses of 1 h duration at 100 lux was administered at Ct 18 evoked delay phase shifts in both the strains, however, the magnitude of the phase shifts was dependent on the altitude of origin. It was 1.4 h Ct in the *haH* strain (Fig. 3.1A) but 10.2 h Ct in the *laH* strain (Fig. 3.1B). Figure 2 also illustrates the free-running rhythmicity following entrainment to LD 12:12 cycles where light pulse administered at Ct 4 did not evoke any phase shifts in the *haH* strain (Fig. 3.2A) but the same pulse evoked advance phase shift of 1.3 h Ct in the *laH* strain (Fig. 3.2B).

Figure 3.3 shows the light pulse PRCs for these strains. Both were ‘weak’ or type 1 PRCs (28), because the magnitude of phase shifts was < 12 h, and the transition from delays to advances was not abrupt. Nonetheless, the *haH* PRC differed from the *laH* PRC in four features. The *haH* PRC had low amplitude as the delay phase shifts were of small magnitude ($\sim$ 1.2 h) while the *laH* PRC had high amplitude as delay phase shifts were of high magnitude ($\sim$ 10 h). The *haH* PRC had A/D ratio > 1 but the *laH* PRC had A/D < 1. The *haH* PRC was characterized by 8 h of dead zone while the *laH* PRC was devoid of it. The cross-over point from delay to advance phase shifts was between Ct 18 and Ct 20 in the *haH* PRC while it was between Ct 22 and Ct 24 in the *laH* PRC. Even the phase shifts at each of 12 phases in the *haH* PRC were significantly less than those of the corresponding phases in the *laH* PRC ($p < 0.01$). Advance phase shifts were
usually followed by two-three transient cycles and shortening of \( \tau \), while delay phase shifts were instantaneous and usually followed by lengthening of \( \tau \).

**DISCUSSION**

The present study demonstrates that the altitude of origin of the haH strain of *D. helvetica*, besides affecting parameters of entrainment and persistent free-running rhythmicity (25, 26), also affected light pulse PRC for the adult locomotor activity rhythm. Altitude of origin similarly affected the light pulse (1 h at 100 lux as used to determine the present PRC) PRC for the oviposition rhythm of the high altitude (HA) strain of *D. ananassae* captured at Badrinath (5,123 m a. s. l.) in the Himalayas (2). The PRC for the HA strain had protracted dead zone and low amplitude which were attributed to the reduced photic sensitivity of the circadian photoreceptors that was acquired through natural selection in response to low environmental temperature at the altitude of its origin (22, 23). Prolonged dead zone and the low amplitude in the PRC of the haH strain of *D. helvetica* also appears to be the result of its reduced photic sensitivity. Evidence for the diminished photic sensitivity of this strain emerged from the previous study which demonstrated that this strain did not become arrhythmic in any of four LD cycles in which photoperiods varied from 10 to 16 h, while the laH strain became arrhythmic in LD cycle with 16 h photoperiod (26). Furthermore, the haH strain required higher intensity of LL (15 lux) to become arrhythmic than that of the laH strain (1 lux). When transferred from LD 12:12 cycles to LL at 1 lux, the haH strain was rhythmic and
its \( \tau \) was unaltered from that in DD, suggesting that LL at 1 lux was apparently perceived as physiological darkness by this strain owing to its reduced photic sensitivity.

Latitude of origin also altered the dead zone and amplitude in the PRCs for the oviposition rhythms of *D. ananassae* strains (29). The PRC for the equatorial *PK* strain (\( 0^\circ \)N latitude) had prolonged dead zone and low amplitude like those of the *haH* strain of *D. helvetica*, whereas the PRC for the *DK* strain (22.29\(^\circ\)N) was devoid of dead zone and had high amplitude like those of the *laH* strain of *D. helvetica*. Dead zone in the PRC of the *PK* strain, however, was attributed to the inadequate strength of light pulses but not to the photic sensitivity. As this strain was evolved in very bright daylight at the equator, with the result it required light pulses of higher strength to evoke phase shifts. Low amplitude of its PRC, in contrast, was ascribed to the rigidity of its pacemaker as its \( \tau \) in DD was conserved closed to 24 h. This strain was evolved in the unique equatorial climatic conditions where natural photoperiod is precisely 12 h throughout the year, thereby eliminating the seasonal variation as observed at higher latitudes. The absence of dead zone and high amplitude in the PRC of the *DK* strain were attributed to its enhance photic sensitivity and broad range of entrainability, respectively.

Reduced photic sensitivity and the resultant low amplitude PRCs are as well reported in few a *Drosophila* mutants isolated by chemical mutagenesis or artificial selection (1, 30). For example, the *late* strain of *D. rajasekari*, isolated through artificial selection for phase angle difference of adult locomotor activity, had delayed \( \Psi_o \), long \( \tau \) and low amplitude PRC (1), similar to that for the *haH* strain of *D. helvetica*. Moreover,
the threshold light intensity of LL to generate arrhythmicity was much higher than that for the wild type. Likewise, the per^I strain of D. melanogaster had long τ and low amplitude PRC for both, the eclosion (30) and adult locomotor activity rhythms (31).

Altitude of origin also altered the A/D ratio in PRCs of D. helvetica strains as the PRC for the haH strain had A/D ratio > 1 but the laH PRC had A/D ratio < 1. The asymmetric PRCs of these strains of D. helvetica might elucidate the process of photic entrainment to 24 h light-dark cycles. The long τ (26.2 h) of the haH strain requires a larger advance portion of its PRC (A/D > 1), whereas the short τ (21.4 h) of the laH strain requires a larger delay portion of its PRC (A/D < 1) for entrainment to 24 h light-dark cycles. This assumption agrees with the model describing the functional significance of the asymmetric PRC for photic entrainment of eclosion rhythm of D. pseudoobscura (32, 33).

This study demonstrates that the altitude of origin profoundly influenced three features of the PRC of the haH strain when compared with those of the laH strain of D. helvetica. The prolonged dead zone and low amplitude in the PRC of the haH strain imply that the sensitivity of the circadian photoreceptors mediating phase shifting response has been diminished owing to the environmental conditions at the altitude of its origin. Strains of Drosophila from the high altitude (23, 29) and latitude (6, 8) have low amplitude PRCs and reduced sensitivity of the circadian photoreceptors.
REFERENCES


Figure 3.1. Double plotted activity record of the representative male of the haH strain (A) and the laH strain (B) of *D. helvetica* entrained by LD 12:12 cycles for the first 8 days and then released in constant darkness (DD) (oblique arrow with DD). Light pulse (LP) of 1 h duration at 100 lux administered at the phase Ct 18 (vertical arrow with LP indicates the beginning of the light pulse) evoked delay phase shifts (-Δθ) of 1.4 h Ct (A) in the haH strain and 10.2 h Ct (B) in the laH strain.

(A) *hah* strain: -Δθ of 1.4 h Ct by LP at Ct 18

(B) *lah* strain: -Δθ of 10.2 h Ct by LP at Ct 18
Figure 3.2. Double plotted activity record of the representative male of the haH strain (A) and the laH strain (B) of *D. helvetica* entrained by LD 12:12 cycles for the first 7 days and then released in DD (oblique arrow with DD). Light pulse of 1 h duration at 100 lux administered at the phase Ct 4 (arrow with LP indicates the beginning of the light pulse) evoked neither advance nor delay phase shift in the haH strain (A) but it evoked advance phase shift (+Δθ) of 1.3 circadian h in the laH strain following two transient cycles.
Figure 3.3. Phase response curves (PRCs) for the adult locomotor activity rhythm of the *haH* strain (curve passing through filled circles) and the *laH* strain (curve passing through open circles). Phase shifts were evoked by light pulses (1 h at 100 lux) administered to 12 phases of the rhythm in DD. Phase shifts (mean ± SD, *N* = 5) are expressed in circadian hours.