Relation of the Circadian System to the Photoperiodic Clock in
*Drosophila triauraria* (Diptera: Drosophilidae):
An Approach from Analysis of Geographic Variation

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(Received 14 February, 1994; Accepted 20 June, 1994)

This paper investigated the relationship between the circadian system and photoperiodic time measurement in different geographic strains of *Drosophila triauraria* which enters reproductive diapause at short daylengths. The critical daylength varied among the geographic strains, about 13 h in the ON (originated from Onuma, 42°N), 12 h in the OI (Oita, 33.2°N) and 10 h in the YK (Yakushima, 30.3°N) strains at 15°C. All the strains exhibited a weak "circadian" oscillation pattern in diapause incidence on NANDA-HAMNER protocol. The dependence of photoperiodic time measurement on cycle lengths close to module τ was shown for the ON and OI strains by experiments using non-24 h light-dark cycles. Thus, no evidence was obtained for covariation between critical daylength and circadian oscillation pattern. The ON and OI strains showed similar patterns of response in the night-interruption experiment, despite the one-hour difference in critical photoperiod.

Key words: photoperiod, diapause, geographic variation, *Drosophila triauraria*

INTRODUCTION

Insects and mites that live in temperate zones frequently enter diapause as an adaptation to a seasonally-changing environment by responding to environmental cues such as photoperiod and temperature, and they often show geographic variation in critical daylength (see reviews, SAUNDERS, 1982; BECK, 1983; TAUBER et al., 1986). However, physiological and genetic bases for the geographic variation have not been well understood.

In many insects and mites, the circadian oscillatory system has been shown to be somehow involved in photoperiodic time measurement (SAUNDERS, 1982). If the circadian oscillatory system itself acts as the photoperiodic clock, critical daylength should covary with some of the circadian parameters. It has been observed that critical daylength covaries with "circadian period" in NANDA-HAMNER protocol in *Pterostichus nigrula* (THELE, 1977) and *Ostrinia nubilalis* (TAKEDA and SKOPIK, 1985) and with τ in the overt rhythm in *Drosophila littoralis* (LANKINEN and LUMME, 1984; LANKINEN, 1986 a, b). However, no evidence for a covariation between critical daylength and any circadian parameters was obtained in *Drosophila auraria* (PITTENDRIGH et al., 1984) and *Tetramychus urticae* (VAZ NUNES et al., 1990). In *Drosophila triauraria*
Bock & Wheeler, critical daylength varies with latitude (Kimura, 1984, 1988 a; Kimura et al., 1993), and photoperiodic time measurement is achieved based on the circadian oscillatory system (Yoshida and Kimura, 1993 a, b). The major objective of this study is to investigate the relationship between the circadian system and critical day-(night-)length for the induction of reproductive diapause using different geographic populations of this species.

MATERIALS AND METHODS

The iso-female lines (i.e., each strain originated from a single field-collected female) of D. triauraria were established from collections made at three localities, Onuma (ON), Oita (OI) and Yakushima (YK), Japan. These strains have been maintained for years on a cornmeal-malt medium (cornmeal 50 g, malt 50 g, sucrose 50 g, dry yeast 40 g, agar 10 g, and propionic acid 5 ml in 1,000 ml of water) under diapause-preventing conditions, 23°C and continuous light (LL).

Individuals were either reared under experimental regimes from the egg stage, or they were reared under LL before eclosion and thereafter under experimental regimes. Glass vials containing the experimental animals and food medium were placed in metal boxes (18 × 9 × 6 cm) in which the light-dark (LD) cycles were administered by electric bulbs controlled by computers (NEC PC-8801 and PC-8001mkII). These boxes were placed in an incubator (SANYO MIR-152) or a temperature-controlled room, and temperature in the boxes was controlled by heater or water current. Experimental temperature was 15 ± 0.5°C and light intensity was 5–50 lux. As an indication of photoperiodic response, the diapause incidence was determined 16 days after eclosion; females with undeveloped ovaries were regarded as being in diapause. About 50 females were examined for each datum point.

RESULTS

Photoperiodic response under 24-h LD cycles

Figure 1 shows the photoperiodic response curves of the three geographic strains under 24 h LD cycles. They were reared under LL until eclosion and thereafter under

![Diagram](image)

Fig. 1. Photoperiodic response curves for the induction of diapause in three geographic strains (ON: 42.0°C, OI: 33.2°C, YK: 30.3°C) of Drosophila triauraria under 24 h light-dark cycles at 15°C.
experimental regimes. Critical daylength (where a 50% reduction of the maximum response occurred) differed among the geographic strains, being about 13 h in ON, 12 h in OI and 10 h in YK. The highest diapause rate also varied among them from 100% in ON to 80% in OI and 45% in YK. In continuous darkness (DD), the incidence of diapause was about 70% in ON, 50% in OI and 0% in YK.

Photoperiodic response under non-24 h LD cycles

To examine the diapause incidence under non-24 h LD cycles, we carried out the experiments with the ON and OI strains under different scotophases ranging from 1 to 14 h, each of which was combined with different photophases extending from 2 to 24 h (Fig. 2). They were reared under LL until eclosion and thereafter under the experimental regimes. When the photophase was 10 or 14 h, the diapause rate abruptly increased as the scotophase was prolonged from 10 to 14 h (Figs. 2 and 3). When the photophase was shorter than 6 h, the response curve became less steep, and the maximal incidence of diapause was observed when T (the length of one light-dark cycle) was about 24 h (for example, with a photophase of 4 h, the peak of diapause incidence appeared when the scotophase was 20 h, or with a photophase of 6 h, it appeared when the scotophase was 16–20 h). When a photophase of 1 h was applied, two peaks were observed in the ON strain; i.e. one appeared when the scotophase was about 12 h and the other appeared when the scotophase was about 22 h.

Resonance experiments

On NANDA-HAMNER protocol, the scotophase was serially extended by intervals of 2–4 h with a constant photophase of 10 h. Figure 3a shows the results when individuals were reared under the experimental conditions from the egg stage to the 16-day adult stage. In the ON strain, a weak resonance effect was observed; slight reductions of diapause were observed when the scotophase was 30 and 50 h. In the YK strain, the incidence of diapause somewhat dropped when the scotophase was about 30 h, and

![Graph showing diapause incidence under different photoperiodic cycles at 15°C.](image-url)
Fig. 3. Diapause incidence in resonance experiments using a constant photophase of 10 h at 15°C. Individuals were reared under the experimental conditions from the egg stage to the 16-day adult stage (a), or they were reared under continuous light before eclosion and thereafter under the experimental regimes (b). ○: ON; ●: OI; ■: YK.

Fig. 4. Night-interruption experiments under 24-h photoperiodic cycles at 15°C; two experimental strains (○: ON; ●: OI) were reared under continuous light or light-dark cycles (LD 10: 14 or LD 6: 18) until eclosion and thereafter under the experimental regimes of LD 10: 14 (a, b) or LD 6: 18 (c, d). The position of point represents the time at which the light pulse of 1 h is initiated in the scotophase.
the second peak was observed when the scotophase was about 42 h. The incidence of diapause was rather constant when the scotophase was extended over 48 h.

When individuals were reared under LL until eclosion and transferred to the experimental regimes on the day of eclosion, the maximal incidence of diapause was observed when the scotophase was 14–16 h in all strains (Fig. 3b). In the cases of the ON and OI strains, the diapause rate dropped somewhat when the scotophase was about 30 h, but was rather constant when the scotophase was extended from 36 h to DD. The diapause incidence was very low in the YK strain. Thus, the diapause rates varied among the geographic strains, but the pattern of resonance response did not.

Night-interruption experiments

Figure 4 shows the effect of night interruption by 1 h light pulses on the diapause incidence over two 24 h light-dark cycles (LD 10:14 and LD 6:18) in the ON and OI strains. They were reared under LL or the LD cycles (LD 10:14 and LD 6:18) until eclosion and thereafter under the experimental regimes. With the shifting of the light pulse from the beginning to the end of the scotophase, the diapause rate initially fell and thereafter rose. The diapause rate was lower in the OI strain than in the ON strain, but the pattern of response to the light pulses did not differ much between these two strains.

DISCUSSION

Critical daylength and diapause rate varied among geographic strains of Drosophila triauraria (Fig. 1). On the other hand, the circadian oscillation pattern was observed for all strains in the resonance experiments (Fig. 3). The influence of the circadian system on the photoperiodic clock was also shown in the OI and ON strains in the experiments with non-24 h light-dark cycles; with a short (1–6 h) photophase, the peak of diapause incidence was observed when \( T \) was about 24 h (Fig. 2). With a photophase of 1 h, a high incidence of diapause was also observed when the scotophase was 12 h in the OI strain. This suggests that this strain recognizes a photoperiod of LD 1:12:1:12 as LD 1:25 (i.e. \( T \) was only 2 h longer than 24 h) by disregarding one of the two photophases (also see Yoshida and Kimura, 1993a). Thus, these strains are assumed to share a common circadian oscillatory system, although they differ in critical daylength. No evidence of covariation between critical daylength and circadian parameters was obtained in D. auraria, a sibling species of D. triauraria (Pittendrigh et al., 1984).

Kimura (1988b) observed that F\(_1\) hybrids between diapausing and non-diapausing strains of D. triauraria have shorter critical daylengths than the parental diapausing strains, and explained the shift of critical daylength in the hybrids based on the assumptions that 1) diapause is determined in reference to the level of hypothetical “diapause-inducing substances” and 2) the rate of accumulation of these “substances” is lower in the hybrids than in the parental diapausing strains. The hybrids therefore require longer nights to enter diapause than the parental diapausing strains, since shorter nights are less effective in inducing diapause in this species (Kimura, 1990). This model is applied to explain the geographic variation in critical daylength and the level of diapause incidence; i.e., the rate of accumulation of “diapause-inducing substances” may be lower in the YK or OI strains than in the ON strain. The former strains therefore
show lower incidence of diapause and also require longer nights to enter diapause. On the other hand, Pittendrigh and Takamura (1993) proposed that the photoperiodic control is only realized within a narrow “window” of temperatures, explaining the latitudinal change of critical daylength in *D. auraria* by a shift of this “window” along the temperature scale.

The above two models can be applied to explain the geographic variation of critical daylength in many insects, but the propriety of these models is still uncertain. In the former model, the existence of “diapause-inducing substances” has not been proved. In the latter model, the physical and biochemical basis of such a “window” is not clear.

ACKNOWLEDGEMENTS

We thank Professor Samuel H. Horii for his advice and encouragement in the course of this study. Our thanks are also due to colleagues in our laboratory.

REFERENCES
