Effects of photoperiod and temperature on reproductive diapause in *Drosophila testacea*

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Summary. In photoperiodic adult diapause of *Drosophila testacea,* the critical daylength fell between 14 and 16 h at 15 °C, but between 13 and 14 h at 18 °C. None entered diapause at LD 10:14 and LL at 23 °C. This species quantitatively responded to daylengths around the critical daylength in the process of diapause induction. The length of the sensitive period seems to change with temperature.

The effect of photoperiods on diapause induction has been considered to be all-or-none; for example, short daylengths induce diapause and long daylengths do not do so at all in long-day insects. However, in recent years, it has been reported that some insects quantitatively respond to daylength in diapause induction and termination[1-3]. Here I report the quantitative response to daylength in reproductive diapause of *Drosophila testacea* van Roser and refer to the effect of temperature on the photoperiodic response.

A strain of *D. testacea* was established by several females collected in Sapporo (43 °N), northern Japan, and maintained for several months under diapause-preventing conditions (23 °C, continuous illumination). In experiments, larvae were reared on yeast medium (dry yeast 8%, sugar 5%, agar 1.4%, propionic acid 0.5%) with mushroom *(Pleurotus cornucopiae)* added, and adults were on the yeast medium alone.

Table 1 gives the effect of photoperiods and temperature. Animals were cultured at various combinations of photoperiods and temperatures throughout their entire life, and the ovarian condition of the females was examined at the age of 16 days after eclosion as an indicator of diapause; females with undeveloped ovaries were recognized to be in diapause[4]. The critical daylength fell between 14 and 16 h at 15 °C, but between 13 and 14 h at 18 °C. At 23 °C, none entered diapause at LD 10:14 (10 h light:14 h dark) and LL (continuous illumination).

To examine the quantitative response to daylengths and the sensitive stages, transfer experiments were carried out (table 2). Animals were first cultured under diapause-preventing conditions, and transferred to diapause-inducing conditions at various ages. Diapause was also examined at the age of 16 days after eclosion. At 15 °C, the transfer from LD 16:8 to LD 12:12 at the age of 8 days after eclosion was sufficient to induce diapause, but the transfer from LD 16:8 to LD 14:10 at the same time was not sufficient. Thus, LD 12:12 is more efficient as a diapause-inducing stimulus than LD 14:10 at 15 °C. The difference was not observed when animals were transferred from LL to these short daylengths. At 18 °C, the transfer from LD 14:10 to LD 12:12 at the age of 8 days after eclosion was sufficient to induce diapause, but the transfer from LD 16:8 to LD 12:12 at the same time was not sufficient. This suggests that LD 16:8 is more efficient to prevent diapause than LD 14:10. Further, LD 16:8 was more efficient to prevent diapause than LL at 15 and 18 °C. Thus, there was a quantitative response to daylengths around the critical daylength.

Temperature seems to change the length of the sensitive period. When animals were transferred from 23 to 18 °C under LD 10:14 at the time of eclosion, most of them entered diapause, but when they were transferred at the age of 4 or 8 days after eclosion, only a few entered diapause. When animals were transferred from LD 16:8 to LD 12:12 at the age of 8 days after eclosion, they entered diapause at 15 °C, but did not at 18 °C. These results indicate that the earlier transfer to short daylengths is needed to induce diapause at higher temperature. This would be due to the fact that the sensitive period ends earlier at higher temperature.

Table 1. Diapause percentages in *Drosophila testacea* under various photoperiodic conditions at 15, 18, and 23 °C. Diapause was examined 16 days after eclosion

	LD 10:14	LD 12:12	LD 13:11	LD 14:10	LD 16:8	LL
15 °C	100.0(27)	100.0(21)	–	87.5(16)	8.3(24)	13.8(29)
18 °C	100.0(24)	95.2(21)	94.6(37)	8.5(94)	0.0(37)	0.0(17)
23 °C	0.0(15)	–	–	–	–	0.0(26)

Numbers in brackets refer to number of females examined.

Table 2. Diapause percentages in *D. testacea* when transferred from diapause-preventing conditions to diapause-inducing conditions at various ages. Diapause was examined 16 days after eclosion

Regime before transfer	Regime after transfer	Time of transfer Adult eclosion	4 days after eclosion	8 days after eclosion	12 days after eclosion
LD 16:8, 15 °C	LD 12:12, 15 °C	–	84.9(53)	78.1(32)	37.5(32)
LD 16:8, 15 °C	LD 14:10, 15 °C	–	84.6(26)	21.9(32)	24.4(41)
LL, 15 °C	LD 12:12, 15 °C	–	90.9(33)	74.1(27)	30.0(20)
LL, 15 °C	LD 14:10, 15 °C	–	79.0(38)	69.6(23)	36.8(57)
LD 14:10, 18 °C	LD 12:12, 18 °C	100.0(25)	88.9(45)	93.9(33)	14.0(43)
LD 16:8, 18 °C	LD 12:12, 18 °C	93.6(31)	63.0(49)	6.4(63)	1.4(73)
LL, 18 °C	LD 12:12, 18 °C	97.2(36)	89.2(37)	37.9(29)	11.5(26)
LD 10:14, 23 °C	LD 10:14, 18 °C	86.7(15)	38.1(63)	9.1(11)	–
LL, 23 °C	LD 10:14, 18 °C	64.7(34)	0.0(18)	2.7(37)	–

Numbers in brackets refer to number of females examined.

It has been well known that temperature modifies the rate of diapause incidence and the critical daylength in photoperiodic diapause of insects. The mechanism by which temperature modifies the rate of diapause has been extensively studied by Saunders[5-7]. He suggested that diapause is induced when a sufficient number of light-cycles were experienced before the end of the sensitive period, and further that the required number of light-cycles to induce diapause is temperature-compensated and the length of the sensitive period is temperature-dependent. He accounted for the lower incidence of diapause at higher temperatures by the interaction between the length of the sensitive period and the summation of light-cycles. For example, higher temperature leads to faster development, hence loss of photoperiodic sensitivity occurs sooner, and the incidence of diapause is reduced owing to the smaller number of cycles experienced. On the other hand, the manner in which temperature affects the critical daylength has been unclear, but it can be explained by the interaction between the temperature-compensated mechanism of the summation of light-cycles and the quantitative response to daylengths. Saunders assumed that long-day cycles are also inductive (in the diapause induction sense) if sufficient of them are seen before the end of the sensitive period[7]. In the case where animals quantitatively respond to daylengths, Saunders' idea can be extended thus; a longer photoperiod requires animals to experience a larger number of light-cycles before the end of the sensitive period to induce diapause. Then, the prolonged sensitive period at lower temperature enables them to enter diapause under a longer photoperiod.

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Liver ferritin synthesis following chronic alcohol administration to rats: Modulation by propylthiouracil

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Summary. Liver ferritin synthesis was inhibited by 22.3% in rats treated with alcohol (2 g/kg) for 45 days. This inhibition was prevented by simultaneous administration (5 mg/kg) of propylthiouracil during the last 15 days. There was no significant effect on liver ferritin concentration.

Ferritin is the major iron-storage protein of mammalian tissues, found predominantly in the liver, spleen and bone marrow[1,2]. In our continuing studies on the effect of alcohol on liver and liver-produced proteins, we have already reported alterations in liver vitamin A stores[3] and plasma protein synthesis[4], and the effect of propylthiouracil (PTU) on these parameters. Here we report a similar effect of alcohol on liver ferritin synthesis and a similar role of PTU.

Materials and methods. Male Wistar rats in 3 groups of 6 animals each (180–220 g) were fed on a 20% protein diet[3], and had constant access to drinking water. Ethanol (2 g/kg) in 20% concentration in sterile saline was injected daily i.p., in 1 intoxicating dose, for 45 days to rats in groups II and III. Rats in control group I received equicaloric glucose by the same route. This caloric intake worked out to about 15% of the total calories consumed by a rat per day. During the last 15 days of the experiment rats in group III received PTU (5 mg/kg) in 20% ethanol. Food consumption of rats in different groups was identical and food was available until the time of sacrifice.

At the end of 45 days each rats was injected i.p. with 10 μCi (370 kBq) of (1-^{14}C)-glycine (9.3 mCi or 344.1 M Bq/mmole from Bhabha Atomic Research Centre, Bombay) per 100 g b.wt, and 120 min after the tracer injection the hepatic portal vein was exposed under ether anaesthesia. The liver was perfused in situ, via the portal vein, with 15–20 ml of ice-cold saline containing 10 mM glycine to flush out blood. The liver was excised, blotted dry, weighed and frozen for ferritin isolation. Ferritin was separated by using a minor modification of the method of Linder and Munro[5]. Ferritin was initially precipitated from the heat-coagulated homogenate-supernatent by ammonium sulphate and the solubilized precipitate was gel-filtered through Sephacryl S-200, superfine (Pharmacia, Uppsala). The protein content of the purified ferritin was quantitated by Lowry's method[6], taking bovine serum albumin as reference standard, since the extinction coefficient of apoferritin is comparable to that of bovine serum albumin[7]. In a separate recovery experiment rat liver ferritin, isolated and purified as described earlier[8], was labelled with ^{125}I (Radiochemical Centre, Amersham) using the chloramine T method[9], and taken through the entire isolation-purification procedure. The recovery averaged 92%. An aliquot of isolated ferritin was counted for ^{14}C in a Nuclear Chicago (720 series) liquid scintillation spectrometer using the dioxane-based scintillator of Geegeebuoy[10]. All the counts were normalized for 80% efficiency. The incorporation of radioactive precursor into ferritin (cpm/mg) was taken as a measure of synthesis[7].

Results and discussion. As shown in the table there was 22.3% inhibition of ferritin synthesis in rats treated chronically with alcohol. Further, this inhibition was prevented by simultaneous PTU administration. The liver ferritin concentration was, however, not significantly affected.

Liver ferritin concentration and ^{14}C-glycine incorporation

Group	Ferritin Concentration (μg protein/g liver	Specific activity (cpm/mg)
I Control	436 ± 45	2939 ± 365
II Alcohol	417 ± 35	2285 ± 167*
III Alcohol + PTU	468 ± 54	3118 ± 539

*$p < 0.02$, the values are mean ± SD for 6 rats.