

TEMPERATURE COMPENSATION OF THE CIRCADIAN OSCILLATION IN *DROSOPHILA PSEUDOOBSCURA* AND ITS ENTRAINMENT BY TEMPERATURE CYCLES*

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Abstract--Detailed analysis of the effects of temperature on the circadian rhythm of eclosion in *Drosophila pseudoobscura* adults yielded results similar to those in similar studies of other poikilotherms : although the period, τ , of the free-running oscillation varies little with different constant temperatures, it can be phase shifted by changes (steps and pulses) and entrained by temperature cycles. Temperature steps-up generate only phase advances ($+\Delta\phi$); steps-down generate only phase delays ($-\Delta\phi$). Pulses, however, give both $+\Delta\phi$ and $-\Delta\phi$. The size of $\Delta\phi$ generated depends on the point in the oscillation's cycle exposed to the temperature signal.

An empirical relationship is found between temperature steps and pulses : to first approximation, the $+\Delta\phi$ or $-\Delta\phi$ generated by 12 hr pulses may be computed from the $\Delta\phi$ generated by the steps comprising that pulse. This finding justifies the assumption that the $\Delta\phi$ normally effected by the step is accomplished rapidly (within a few hours).

An empirical response curve model for (square wave) temperature cycle entrainment of the eclosion rhythm is developed: from the $\Delta\phi$ response curves for single 12 hr pulses (high or low), the phase angle difference, Ψ , between 24 hr recurrent pulses (square-wave forcing oscillation) and the eclosion rhythm (driven circadian oscillation) is correctly predicted.

From the response curves for a temperature step-up (T_1/T_2) and a temperature step-down (T_2/T_1) the $\Delta\phi$ response curves for single pulses ($T_1/T_2/T_1$) of different duration were computed. These computed response curves differ in their zero phase shift points; that is, exposure of certain points in the oscillation's cycle to a temperature step-up will generate a $+\Delta\phi$ equal to the $-\Delta\phi$ generated by the step-down which comes n hr later, n being the duration of the pulse.

Assuming the validity of the empirical response curve model for temperature entrainment, the variation in zero phase shift points of the computed pulse response curves predicts a parallel variation of Ψ between the eclosion peak and 24 hr recurrent pulses of different durations (different thermoperiods). Such an experiment thus tests both the validity of deriving pulse response curves from

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step response curves and the validity of the response curve model of entrainment ; and indeed the agreement between observation and prediction is close.

Most workers agree that this remarkable conjunction of properties--relative invariance of τ with different constant temperatures and entrainment by temperature cycles--implies some mechanism for temperature *compensation* of circadian oscillations. Possible mechanisms of temperature compensation are discussed, and a 'temperature adaptation' model--based on feedback inhibition --is proposed which readily describes the known effects of temperature on circadian oscillations. Present evidence does not exclude other possible mechanisms; the present model is intended only to show that what is known can be readily simulated by assuming a mechanism whose elements--oscillations and temperature adaptation--are known to occur in living systems.

INTRODUCTION

ONE OF the most striking properties of circadian oscillations is that, even in poikilotherms, their free-running period (τ) is essentially invariant through a wide range of constant temperatures. On the other hand, such oscillations in poikilotherms are readily entrained by temperature cycles. The essential invariance of τ does not therefore imply a true temperature independence or temperature insensitivity; it must derive from a temperature compensation mechanism in the oscillatory system (BRUCE and PITTENDRIGH, 1956; HASTINGS and SWEENEY, 1957; PITTENDRIGH, 1960 ; RAWSON, 1960). Compensation as against outright independence is also implied by the fact that the invariance of τ with temperature is rarely, if ever, absolute; sometimes the slight dependence reveals a Q_{10} less than 1.0--in other words, slight overcompensation (HASTINGS and SWEENEY, 1957).

Sensitivity of the oscillation(s) to temperature *change* has been demonstrated in a diversity of ways in several organisms. Several workers have reported that phase advances (but not phase delays) are generated by temperature steps-up, and that the magnitude of the advance (+ A) is a function of the phase of the steady-state oscillation at which the step is applied (BUNNING and TAZAWA, 1957; EHRET, 1959; SWEENEY and HASTINGS, 1960; MOSER, 1962). It is similarly reported that temperature steps-down generate phase delays ($-\Delta\phi$) whose magnitude is again a function of the steady-state phase to which it is applied; steps-up never generate phase delays (MOSER, 1962; WILKINS, 1965). Temperature pulses, on the other hand, cause both phase delays ($-\Delta\phi$) and phase advances (+ $\Delta\phi$); the magnitude and sign of the phase shift is a function of the steady-state phase perturbed by the pulse (LEINWEBER, 1956; BUNNING and TAZAWA, 1957; BALL and DYKE, 1958; SWEENEY and HASTINGS, 1960 ; RUDDAT, 1961; MOSER, 1962 ; ROBERTS, 1962 ; WILKINS, 1962, 1965).

The present paper reports in some detail comparable results for the circadian oscillation in *Drosophila pseudoobscura* adults and pupae* (cf. PITTENDRIGH, 1966), and develops an empirical model of entrainment by temperature cycles based on

* Adult drosophilids exhibit the eclosion rhythm. The circadian oscillation which gates this adult act is also present in the pharate adult stage before the emergence of the adult. Throughout we have referred to all stages enclosed by the puparium as pupae but these of course include both the pupa itself and the pharate adult.

the $\Delta\phi$ response curves. It then discusses briefly the bearing of this model on proposed temperature compensation mechanisms and develops an explicit mathematical basis for the empirical model.

MATERIALS AND METHODS

Automatic devices, incorporating a rigorously controlled light and temperature environment, have been developed in the Princeton University laboratory for the assay of the time of emergence of adult drosophilids from populations of pupae (described in ZIMMERMAN, 1966). Pupae are reared in plastic boxes in an LD 12 : 12 light/dark cycle at constant temperature (20 or 28°C). They are harvested by a flotation method and glued to a small brass 'holding' plate which is bolted to the face of a precisely temperature-controlled brass 'mounting plate'. The pupae on the holding plate are enclosed by a Lucite cover with a tapered base that vents above a vial of detergent solution. The mounting plate is suspended from a solenoid which is activated every 30 min, lifting and dropping (against a rubber stop) the system and thus shaking out, into the vial of detergent, all flies which have emerged in the previous 30 min interval. The vials of detergent are assembled in a circular tray which is rotated--1 vial/hr--by a spring-loaded escapement mechanism. The trays of twenty-four vials are changed daily, and the counts of flies in the hourly collection yield an assay of the eclosion rhythm.

Light is provided by a water-cooled 4 W fluorescent bulb that delivers 100 ft-c. at the surface of the pupal mounting plate; the lamps are connected to time switches. Temperature control of the heavy brass *mounting* plate is achieved by circulating water through milled channels within them from a precisely temperature-controlled water-bath. The mounting plates are actually coupled by three-way solenoid water valves to two water-baths at different temperatures; time switches actuate the water valves and thus switch water flow from one bath to the other. Variations in temperature on the holding plate surface are less than 0.1°C, and temperature changes of 10°C are accomplished within 50 sec.

Pupae are glued to the holding plate during a regularly phased light (12 hr) period, at the end of which they are released into constant dark (DD). The last LD cycle is defined as Day 0 of the experiment. Day 1 is the first full cycle in DD, Day 2 the second, and so on. At 20°C populations of pupae continue to yield emergences up to Day 7.

The Circadian Time, or CT scale, running from CT 0 to CT 24 (hr), denotes the organism's subjective time; it measures the full cycle of change involved in the oscillation whose period, τ , is thus always normalized to 24 hr (PITTENDRIGH and MINIS, 1964).

The times at which temperature steps or pulses are applied to the *Drosophila* pupae can only be rigorously specified in terms of circadian time. However, the correspondence of Zeitgeber Time (ZT) to circadian time is sufficiently close (it is not exact) during the initial stages of a DD free run following LD 12 : 12 to justify utilization of ZT as an operational measure of CT.

**RESULTS: THE EFFECTS OF TEMPERATURE ON THE
DROSOPHILA PSEUDOOBSCURA OSCILLATION**

Small dependence of τ on temperature

Table 1 shows that the dependence of τ of the free-running oscillation in *D. pseudoobscura* is extremely small: at 10°C it is 24.7 hr, at 20°C it is 24.0 hr, and at 28°C it is 23.7 hr ($Q_{10} = 1.02$).

TABLE 1--EFFECT OF TEMPERATURE ON THE FREE-RUNNING PERIOD, τ , OF THE *Drosophila* ECLOSION RHYTHM

Temperature of free run (°C)	10		20		28	
Rearing temperature (°C)	20		22		20	
Treatment before DD release	6 days at 10°C		0.5 days at 20°C		4 days at 26°C 2 days at 28°C	
Day	Median*	τ	Median†	τ	Median:	τ
1	3.4	25.5	3.4	23.9	2.8	24.3
2	4.9	23.7	3.3	24.2	3.1	23.3
3	4.6	24.8	3.5	24.3	2.4	24.1
4	5.4	25.0	3.8	23.9	2.5	23.9
5	6.4	24.3	3.7	24.0	2.4	23.0
6	6.7	24.9	3.7	23.6	1.4	
7	7.6	24.9	3.3	24.4		
8	8.5	23.8	3.7			
9	8.3	25.6				
10	9.9					
	Mean $\tau = 24.7$		Mean $\tau = 24.0$		Mean $\tau = 23.7$	

* Data pooled from two identical experiments.

† Data pooled from twelve identical experiments : average standard error of medians per day was ± 0.29 hr (PITTENDRIGH, 1966).

‡ Data pooled from three identical experiments.

Entrainability to temperature cycles

Fig. 1 shows the phase of six rhythms during and after exposure to six temperature cycles (12 hr, 28°C/12 hr, 20°C) whose phases relative to EDT were all different. It is clear: (1) that the Ψ of the rhythm relative to the temperature cycle is the same ($\Psi = +2.5$ hr); and (2) that Ψ of the free-running rhythm (relative to the prior temperature cycle) is also constant. In short, the temperature cycle does indeed entrain the phase of the driving oscillation underlying the rhythm.

Temperature steps

Fig. 2 summarizes in $\Delta\phi$ response curves the steady-state phase shifts (cf. PITTENDRIGH, 1966) generated by (1) temperature steps-up 20/28°C) and (2)

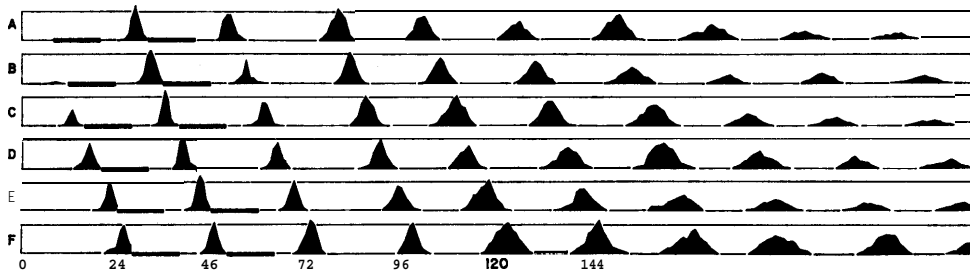


FIG. 1. Distribution of eclosion peaks during and after entrainment of six *D. pseudoobscura* cultures to a 24 hr temperature cycle (12 hr, 28°C/12 hr 20°C) in constant dark (DD). The phase of the temperature cycle was systematically varied with respect to local EDT. The last two pulses of 12 hr at 28°C are shown as heavy bars. Hour 0 on the time scale corresponds to midnight of the first day of collection.

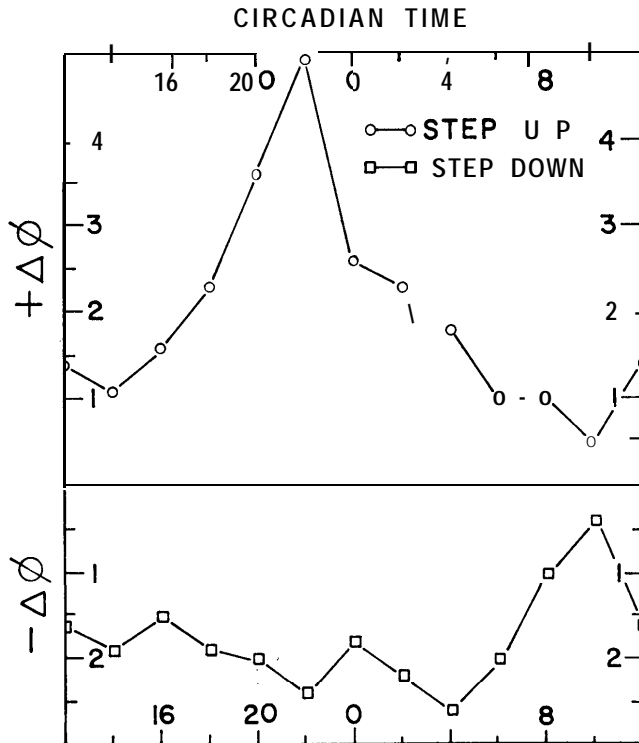


FIG. 2. Phase shift response curves for temperature steps-up (20/28°C) and temperature steps-down (28/20°C). Pupae were systematically exposed to temperature steps during the first 24 hr of DD free run (CT 12 of Day 0 to CT 12 of Day 1) after prior entrainment to an LD 12 : 12 cycle at constant 20 or 28°C. $\Delta\phi$ values for temperature steps-down are the average of $\Delta\phi$'s (median of experimental minus that of free-run control) for Days 6, 7, and 8; for temperature steps-up, the $\Delta\phi$ values are the average of $\Delta\phi$'s for Days 4 and 5.

temperature steps-down (28/20°C). It will be noted that: (1) steps-up generate only $+\Delta\phi$; (2) steps-down generate only $-\Delta\phi$; (3) that the magnitude of the $\Delta\phi$ is a function of the phase (CT point) of the oscillation exposed to the step. The amplitude of the step-up curve is greater than that for the step-down; there is a strong suggestion that the minima for both $+\Delta\phi$ and $-\Delta\phi$ occur at the same phase (CT 10) of the oscillation; otherwise there is no symmetrical relationship between the two curves.

Temperature pulses

Fig. 3(A), (B) summarizes in A+ response curves the steady-state phase shifts generated by 12 hr high and low temperature pulses (HTP, 20/28/20°C; LTP, 28/20/28°C). Both HTP'S and LTP'S generate $+\Delta\phi$ and $-\Delta\phi$; the sign and magnitude of $\Delta\phi$ is a function of the circadian time. The maximum $+\Delta\phi$ is greater than the maximum $-\Delta\phi$. For HTP'S, $+\Delta\phi$'s are generated between CT 17 and CT 5; for LTP'S, $+\Delta\phi$'s are generated between CT 5 and CT 17.

TOWARDS AN EMPIRICAL MODEL FOR TEMPERATURE EFFECTS

The derivation of pulse response curves from step response curves

It is, of course, not clear, *a priori*, that the $\Delta\phi$ action of steps is completed rapidly; and if it is not, there will be no simple way of deriving the pulse curve from the two step curves. However, if the effect by a given temperature step is in fact completed rapidly, the phase shift effected by a given pulse (up or down) will be computable (to a first approximation) from the $\Delta\phi$ curves for the two steps which compose the individual pulse.

Table 2 shows computations of the net $\Delta\phi$ effect of the two steps (20/28°C and 28/20°C) which comprise a 12 hr pulse (20/28/20°C or 28/20/28°C). Values are given for both HTP'S and LTP'S applied systematically across the circadian time scale (i.e. initiated systematically at phase points that scan the full cycle of the oscillation). These **predicted** values for $\Delta\phi$ generated by pulses (both HTP and LTP) are plotted as dashed lines for comparison with the **observed** $\Delta\phi$ response curves for pulses in Fig. 3(A), (B). There is a reasonably good conformity of prediction to observation. For HTP'S the agreement is very good. We conclude that the $\Delta\phi$ of a single temperature change (step-up or -down) is indeed effected promptly--at least within the 12 hr necessary to make the computations of Table 2 even approximately correct.

The Ψ of the entrained steady state

Given this encouragement that the $\Delta\phi$ action of temperature change is effected in a short time, we now attempt to discuss the mechanism of entrainment by temperature cycles in a fashion comparable to that developed by PITTENDRIGH and MINIS (1964) for entrainment by light cycles. The essential feature of the empirical model developed by PITTENDRIGH and MINIS (1964) is the assumption that the $\Delta\phi$ action of a light pulse is (a) effected essentially instantaneously and (b) is properly described by the $\Delta\phi$ response curve for single pulses applied to a free-running rhythm. In the entrained steady state the period (τ) of the free-running

oscillation is changed to match precisely the period (T) of the entraining light cycle, and a determinate phase relation (Ψ) is developed between the oscillation and its driving cycle. Entrainment is effected by a discrete $\Delta\phi$ in each cycle which, as it were, corrects the discrepancy between τ and T: in brief, in the entrained steady state :

$$\tau - T = \Delta\phi_{ss}$$

$\Delta\phi$ is, of course, generated only by a pulse falling at a unique phase point in the (driven) circadian oscillation. Thus the oscillation in the entrained steady state assumes a definite phase relation (Ψ) to the cycle of entraining pulses. PITTENDRIGH and MINIS (1964) and PITTENDRIGH (1966) show that this empirical model accounts for a very wide range of the phenomena of entrainment by light pulses.

TABLE %-DERIVATION OF $\Delta\phi$ RESPONSE CURVES FOR 12 hr HTP AND LTP FROM RESPONSE CURVES FOR TEMPERATURE STEPS-UP AND -DOWN

12 hr HTP (20/28/20°C)					12 hr LTP (28/20/28°C)				
CT of step-up	$\Delta\phi$	CT of step-down	$\Delta\phi$	Sum, or net Ad	CT of step-down	$\Delta\phi$	CT of step-up	$\Delta\phi$	Sum, or net $\Delta\phi$
0	+2.6	14.6	-1.8	+0.8	0	-1.8	10.2	+0.6	-1.2
2	+2.4	16.4	-1.6	+0.8	2	-2.2	11.8	+1.3	-0.9
4	+1.8	17.8	-1.8	—	4	-2.6	13.4	+1.2	-1.4
6	+1.0	19.0	-2.0	-1.0	6	-2.0	16.0	+1.6	-0.4
8	+1.0	21.0	-2.2	-1.2	8	-1.0	19.0	+3.0	+2.0
10	+0.5	22.5	-2.3	-1.8	10	-0.4	21.6	+4.6	+4.2
12	+1.4	1.4	-2.1	-0.7	12	-1.6	22.4	+4.2	+2.6
14	+1.1	3.1	-2.4	-1.3	14	-1.9	0.1	+2.6	+0.7
16	+1.6	5.6	-2.1	-0.5	16	-1.5	2.5	+2.2	+0.7
18	+2.3	8.3	-0.9	+1.4	18	-1.9	4.1	+1.7	-0.2
20	+3.6	11.6	-1.3	+2.3	20	-2.0	6.0	+1.0	-1.0
22	+4.9	14.9	-1.7	+3.2	22	-2.3	7.7	+1.0	-1.3

In deriving the 12 hr pulse response curves from the step-up and -down response curves it was assumed that the phase shift resulting from the first step had been accomplished by the time of the second step. The phase shift resulting from the first step was thus added to the duration of the pulse, 12 hr, to determine at what CT point the second step came (see text).

For purposes of first approximation in the computations we ignore the 0.3 hr difference between τ at 20°C and at 28°C; it is taken as $\tau = 24$ hr. Following the approach developed for light pulses, we note that in the entrained steady state the $\Delta\phi$ generated by the recurrent temperature pulse must be zero:

$$\tau - T = \Delta\phi_{ss}; \quad 24 - 24 = 0.$$

Inspection of the $\Delta\phi$ response curves for 12 hr HTP's and LTP's (Fig. 3) shows that $\Delta\phi = 0$ occurs at two distinct circadian times (phase points in the oscillation).

For HTP's these are CT's 17 and 5; for LTP's they are nearly the same, as they theoretically should be. It thus appears that two distinct phase relations (Ψ_A and Ψ_B) are possible between the circadian oscillation and the 24 hr cycle of 12 hr temperature pulses; that is, with the step-up occurring at either CT 16 or CT 6 (Fig. 4). However, only one of these phase relations is stable. Consider the effect of a minor perturbation on Ψ_A such that the circadian oscillation is slightly phase advanced (Case 1 in Fig. 4) in one cycle when the step-up occurs; it falls, for

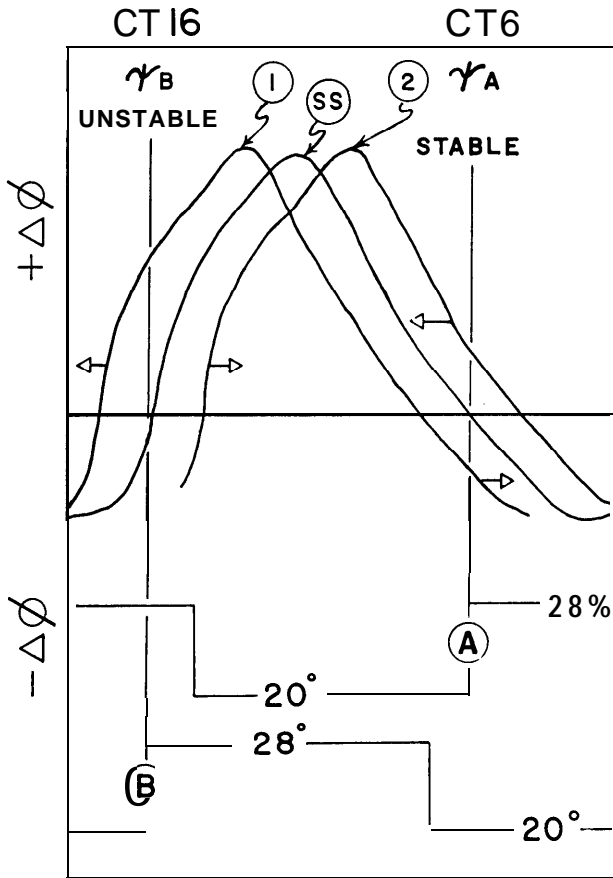


FIG. 4. The existence of two phase relations (Ψ_A and Ψ_B) between oscillation and temperature cycle at which $\Delta\phi = 0$. Only Ψ_A is stable. See text.

example, on CT 8. The effect is to phase delay the oscillation back towards its equilibrium position. Similarly, a minor instability (Case 2 in Fig. 4) resulting in phase delay of the oscillation (relative to the temperature cycle) will cause the step-up to fall on the phase advance part of the response curve, again driving the oscillation back toward its equilibrium position. On the other hand, comparable

instabilities with respect to Ψ_B become amplified in the same direction in successive cycles; the system is thus shifted away from Ψ_B to the stable condition at Ψ_A .

The model thus predicts a unique phase relation between the circadian oscillation (of pupal eclosion) and the temperature cycle; the median of the eclosion peak is taken as the phase reference point of the oscillation, and it marks CT 3.3 (PITTENDRIGH and MINIS, 1964). That point is predicted to fall 2.7 hr before the step-up initiating the high temperature half of the cycle ($\Psi_A = +2.7$ hr, predicted).

Table 3 summarizes observed values of Ψ for cycles of 12 hr, 28°C/12 hr, 20°C. The six cultures in column 1 were raised in LD 12 : 12 at 20°C and on transfer to

TABLE 3-Y FOR CYCLES OF 12 hr, 28°C/12 hr, 20°C

1		2	
Culture	Ψ'	Culture	Ψ'
1	+2.1	1	+2.8
2	+2.2	2	+2.7
3	+2.0	3	+2.6
4	+2.0	4	+2.8
5	+1.8	5	+2.8
6	+1.8	6	+2.7
Mean Ψ'	= +2.0	Mean Ψ'	= +2.7

The phase-relation (Y) between the eclosion rhythm and an entraining temperature (12 hr, 28°C/12 hr, 20°C). Ψ' (measured in hr) is the interval between the eclosion median and the temperature step-up. The cultures in column 1 were raised in LD 12 : 12 at 20°C before being transferred to DD and the initiation of the temperature cycle; the first high pulse began at CT 00 of the first DD day. Cultures in column 2 were raised in LD 12 : 12 at 28°C before being transferred to DD and the initiation of the temperature cycle; the first low temperature pulse began at CT 20 of the first DD day.

DD were subjected to an initial HTP; the six cultures in column 2 were raised in LD 12 : 12 at 20°C and on transfer to DD were subjected to an initial LTP. It is evident that the Ψ' observed is essentially the same in all cases (for initial HTP, $\Psi' = +2.0$; for initial LTP, $\Psi' = +2.7$) and a good approximation to the predicted value ($\Psi' = +2.7$ hr).

Ψ' as a function of thermoperiod

A family of $\Delta\phi$ response curves for several HTP's (20/28/20°C) of various durations has been computed; they were derived in the same way as the computed curves in Fig. 3 (from the step $\Delta\phi$ response curves). We can, using these curves, predict the dependence of Ψ' on the duration of the HTP repeated at 24 hr intervals; that is, the dependence of Ψ' on the duration of the 24 hr thermoperiod cycles.

Fig. 5 compares these predictions with observed values of Ψ . There is a good measure of agreement for both observed and computed values; Ψ shifts from being slightly positive to negative as thermoperiod increases.

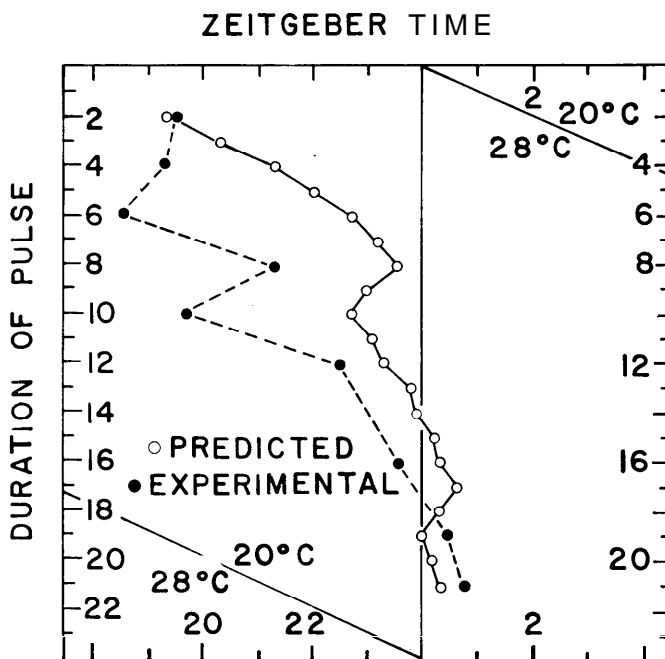


FIG. 5. Predicted and observed values of Ψ (the phase relationship between rhythm and temperature cycle) as a function of thermoperiod (the duration of the high temperature pulse in each 24 hr cycle). Zeitgeber time is given in hours; ZT 0 marks the beginning of the high temperature pulse.

TOWARDS A THEORETICAL MODEL

The previous paragraphs demonstrate (1) that the phase-shifting action of pulses is empirically the sum of their constituent steps; and (2) entrainment by square-wave temperature cycles can be treated empirically as a recurrence of discrete phase shifts induced by the high (or low) pulse occurring in each cycle. Do these empirical exercises afford any basis for development of a theoretical model?

Discussion of the essential invariance of τ through a range of constant temperatures has, for the most part, only agreed that some form of temperature compensation, rather than temperature insensitivity, is involved. To the best of our knowledge, only two qualitatively defined models for compensation have been so far advanced. PITTENDRIGH (1958) suggested that compensation of the driving system could derive from its being in fact composed of two coupled oscillations with complementary temperature dependence of period. BUNNING (1964) discusses

an alternative approach: that of one oscillation composed of two reactions (half-cycles) having opposite (complementary) temperature coefficients such that a higher temperature would shorten the first half to the same extent that it would lengthen the second half of the cycle.

The data summarized in Fig. 6 militate strongly against this approach. The figure presents $\Delta\phi$ response curves for 15 min light pulses (100 ft-c. white fluorescent) applied to the free-running rhythm of *D. pseudoobscura* pupae at three different constant temperatures (10, 20, and 26°C). As discussed in PITTENDRIGH

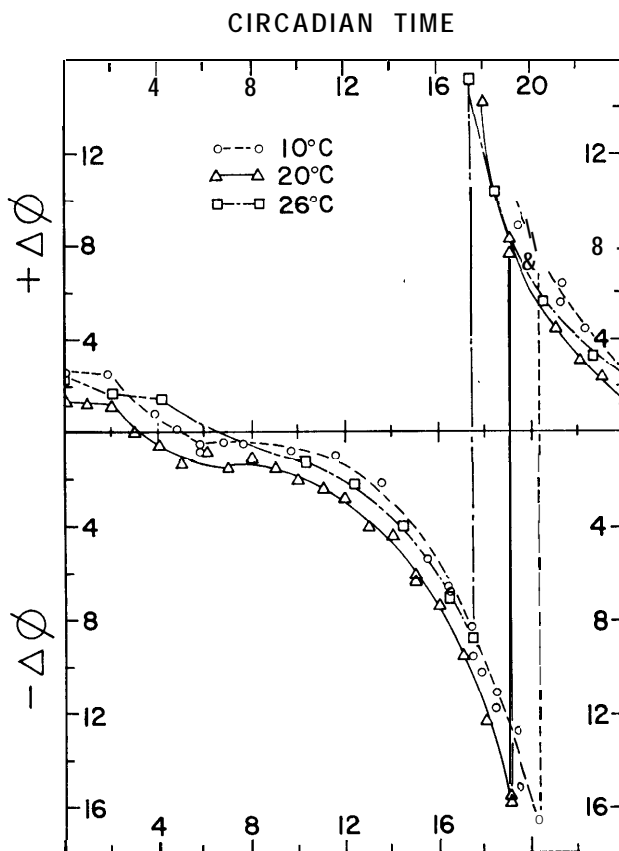


FIG. 6. $\Delta\phi$ response curves for 15 min light pulses (100 ft-c., white fluorescent) applied to the free-running rhythm of *D. pseudoobscura* pupae at three constant temperatures, 10, 20, and 26°C. Pupae were raised in LD 12 : 12 at constant 10, 20, and 26°C, released into DD (Day 0) and exposed to single 15 min light pulses at CT's 0 to 23 of Day 1. The phase shift values ($\Delta\phi_{ss}$) are the average of $\Delta\phi$ values for the last 2 to 3 days of eclosion, by which time transients have subsided (cf. PITTENDRIGH and MINIS, 1964). Vertical lines cross through those CT points where exposure to the pulse caused equal numbers of flies to reset via advance and delay. The plots of the 10 and 26°C response curves are normalized to the free-running period, τ , at the respective temperatures.

(1966) the $\Delta\phi$ response curve is, of course, a function and measure of the wave form of the driving oscillation in the system. The minor differences among the response curves in Fig. 6 indicate *that wave form as well as period is essentially invariant with temperature*, and this result is incompatible with temperature compensation models of the type Bunning discusses. Also incompatible with this class of model is the general result that steps-up generate only $+\Delta\phi$ and steps-down only $-\Delta\phi$.

A third very general approach to the problem is to submit the circadian output of a temperature-dependent oscillation to feed-back inhibition by another temperature-dependent reaction whose product is Y. The time constants in such a model system can be written to effect feed-back compensation in a matter of hours or less. Temperature adaptation of rate constants in poikilotherms is in fact known to develop very rapidly in several cases (BULLOCK, 1955; PROSSER, 1958). Such a property may be incorporated into a model previously developed for the light-sensitive oscillation in *Drosophila pseudoobscura* pupae ; the model is described by two equations:

$$dr/dt = f(s,r) \quad (1)$$

and

$$ds/dt = g(s,r), \quad (2)$$

s and r are state variables, and f and g are specific functions detailed elsewhere (PAVLIDIS, 1967). We now introduce temperature compensation by assuming that temperature affects only the rate of s production, and that this effect is opposite to that of the compensatory process. Thus, equation (1) can be rewritten as :

$$ds/dt = g(s,r) + h(s) - F(T, Y), \quad (3)$$

where h(s) is a function of s only, and F(T, Y) depends on the temperature, T, and an inhibitory factor Y.

For *constant* temperature, Y has such a value as to make $F(T, Y) = 0$, thus reducing equation (3) to equation (1) ; that is, ds/dt depends only on $f(s,r)$ at different constant temperatures, and τ remains essentially invariant. However, when T *changes* (step) we assume that Y also changes, eventually reaching a new steady-state value making $F(T, Y) = 0$. Referring to Fig. 7, we incorporate known temperature effects by assuming that Y changes in the following manner: if T is an increase (step-up), F(T, Y) is transiently positive, thus allowing a temporary increase (acceleration) in ds/dt . Similarly, if T is a decrease (step-down), F(T, Y) is transiently negative ; a temporary slow down (deceleration) of ds/dt results. Transient increases and decreases in ds/dt cause, of course, $+\Delta\phi$'s and $-\Delta\phi$'s, respectively, of the oscillation.

Another experimental finding which must be accounted for in the theoretical model is that greater phase shifts result from temperature steps-up than from steps-down (using the same step sizes). Since the nature of Y is unknown at present, we can simplify its formalization by describing Y as 'equivalent temperature' and by assuming that the system is sensitive to T- Y only.

A specific form of $F(T, Y)$ and an equation for the variation of Y satisfying the above requirements are given by :

$$F(T, Y) = \exp ((T - Y/C) - 1) \quad (4)$$

and

$$t_1(dy/dt) + Y = T, \quad (5)$$

C is a temperature scale factor and t is a constant determining the time for $F(T, Y)$ to return to 0 after a step; that is, t determines the length of the 'lag' in Fig. 7.

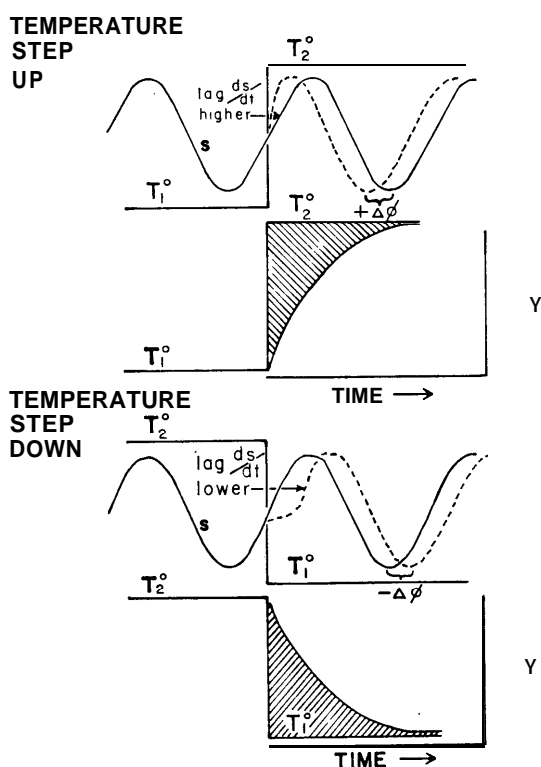


FIG. 7. Response of the model temperature-compensated oscillation to a temperature step-up (T_1/T_2) and step-down (T_2/T_1). An assumed time 'lag' between the response of the temperature dependent oscillation (output s) and the reaction producing inhibitor (Y) allows temporary changes in ds/dt ; such changes in ds/dt cause, in turn, phase shifts of the oscillation. A time constant, t_1 , determines the length of the time lag; the resultant differential between ds/dt and dY/dt during this lag is shown by cross-hatching.

A detailed discussion of the theoretical model together with computer simulation results appears elsewhere (PAVLIDIS *et al.*, 1968).

In effect, then, the $\Delta\phi$ generated in the model system by temperature steps (and hence pulses) reduces to the fact that temperature adaptation requires a matter of some hours to complete: when it is completed, the period and wave form of the oscillation assume their temperature-invariant form.

It has been our purpose only to show that a mechanism adequate to describe all known effects of temperature on circadian oscillations can be constructed on the basis of a simple feed-back-regulated oscillation which is itself temperature dependent. We have not yet explored the possibility that a system of coupled oscillators with complementary temperature dependencies could also accommodate all of the known generalizations; and **a fortiori** we cannot judge whether, if it can, it provides a more attractive basis for further work.

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