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Van D. Gooch

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Abstract: A mathematical model has been examined that attempts to mimic the effects of changes in environmental conditions on circadian rhythms. The basis of the model claims that for a given set of environmental conditions (e.g., light, temperature and chemical concentrations) there exists a limit cycle that has a given position. When an environmental treatment is applied that is different from the control conditions, the position of the new limit cycle changes and the oscillating parameters of the circadian system are now attracted toward this newly positioned limit cycle. If conditions are subsequently returned back to control levels, the control limit cycle again takes effect and the displaced parameters are attracted back to the postion of control limit cycle. The model provides a description of what happens as a result of a pulse of new environmental conditions as well as what happens while the new conditions are in effect. Actual results involving entrainment, phase-release, and pulse experiments are compared to modeled results and a positive correlation is seen. Equations in closed form have been developed from the model that describe release-assay curves and phase response curves (including the transition between type 1 and type 0 behavior). Presumably a change in environmental conditions changes several aspects of a circadian rhythm limit cycle, but this work suggests that most of the features of a circadian rhythm experiment can be qualitatively mimicked by simply shifting the position of the limit cycle relative to new environmental conditions.

Key Words not in the Title: *Neurospora*, van der Pohl Equations, oscillations, phase response curves, entrainment, transients, induced-phase, zeitgeber

Introduction:

For many years the limit cycle concept has been used to help describe circadian oscillatory phenomena (Pavlidis, 1973; Winfree, 1980; Taylor et al., 1982; Winfree, 1986; Carpenter and Grossberg, 1987). It is well known that circadian systems can be perturbed by changing the light, temperature, or chemical environmental condition for a short period of time and then returning the organism to control conditions (a **pulse** experiment). When the rhythm is measured under control conditions after the pulse, it is frequently found that the rhythm returns to the same wave form with the same amplitude but with a different phase compared to an control that did not receive the pulse. This characteristic fits nicely with the concept of a limit cycle (i.e., the treatment moves the oscillating parameters off of the original limit cycle, and then when returned to the original conditions the parameters will asymptotically return to the original limit cycle pattern but with a new phase). Limit cycles also have an unusual characteristic known as a **singularity** (Minorsky, 1962; Pavlidis, 1973). A singularity is a unique point that, if attained, will cause the oscillation to stop. However if the singularity point is not precisely reached, there will initially be a small amplitude rhythm and the amplitude will increase on each successive cycle until the rhythm eventually goes back to its original wave form and shape corresponding to its limit cycle. Several experiments have been performed with circadian rhythms that are consistent with the singularity concept (Engelman et al., 1978; Peterson, 1980b; Winfree, 1980; Peterson, 1981; Taylor et al., 1982, and Gooch et. al. 1992).

More recently, questions have been asked regarding what happens during the time a pulse is being applied to a circadian system. Pavlidis (1973) proposed and Peterson and Saunders (Peterson, 1980a; Peterson and Saunders, 1980) developed the idea that the new environmental conditions during a pulse would invoke a new limit cycle with different equation constants, shape, and rates from the limit cycle under normal control conditions. This only stands to reason, given that limit cycles in living systems would be caused by the rate kinetics of cellular/biochemical processes, and undoubtedly some of these processes are being affected by the

treatment in question. Thus, as a light or temperature pulse is being applied a corresponding new limit cycle would be created and the oscillating parameters would seek this new limit cycle (Winfree (1980) often uses the term 'attracting cycle' which has some valuable connotations). As soon as the pulse treatment is removed, the original limit cycle would again come into effect, but by this time the oscillating parameters would be far displaced from the original limit cycle. This model not only explains how oscillating parameters can be displaced during a pulse, but it is also consistent with other circadian rhythm phenomena (Pavlidis, 1973; Peterson, 1980a; Peterson and Saunders, 1980). In this paper, an adapted form of the Peterson limit cycle model will be used to demonstrate the phenomena of entrainment, light induced phase, and phase response curves. An equation in closed form is presented that represents such phenomena.

In Peterson's model, a treatment of new environmental conditions was allowed to affect both the radius and the position of the limit cycle (Peterson, 1980a). The model presented here is a simplified version of Peterson's model in that only the position of the limit cycle is altered as a function of new environmental conditions. This model will be referred to as the **displacement limit cycle model** and has the following features:

a. The only effect a treatment of a set of new environmental conditions is allowed to have is to shift the position of the limit cycle on a two dimensional phase plane plot.
b. The limit cycles are perfectly circular with perfectly linearly radiating isochrons.
c. The period length under constant conditions (free running period length) is set to be the same at any light intensity, temperature or other environmental condition (if an actual value for the free running period is used in this manuscript, it is 22 hours to be roughly consistent with the *Neurospora*'s free running conidiation rhythm period length, the biological system the author most commonly uses).

It must be noted that this model is extremely simple and surely too simplistic, but it is important first to investigate the degree of predictability of such a simple model, then, if need be, more sophisticated features may be added. It is clear that actual circadian rhythm limit cycles would be influenced in size, shape, and timing by changing environmental conditions, but it is the goal of this manuscript to isolate the single influence of limit cycle repositioning and to examine to what extent it can describe circadian rhythm phenomena.

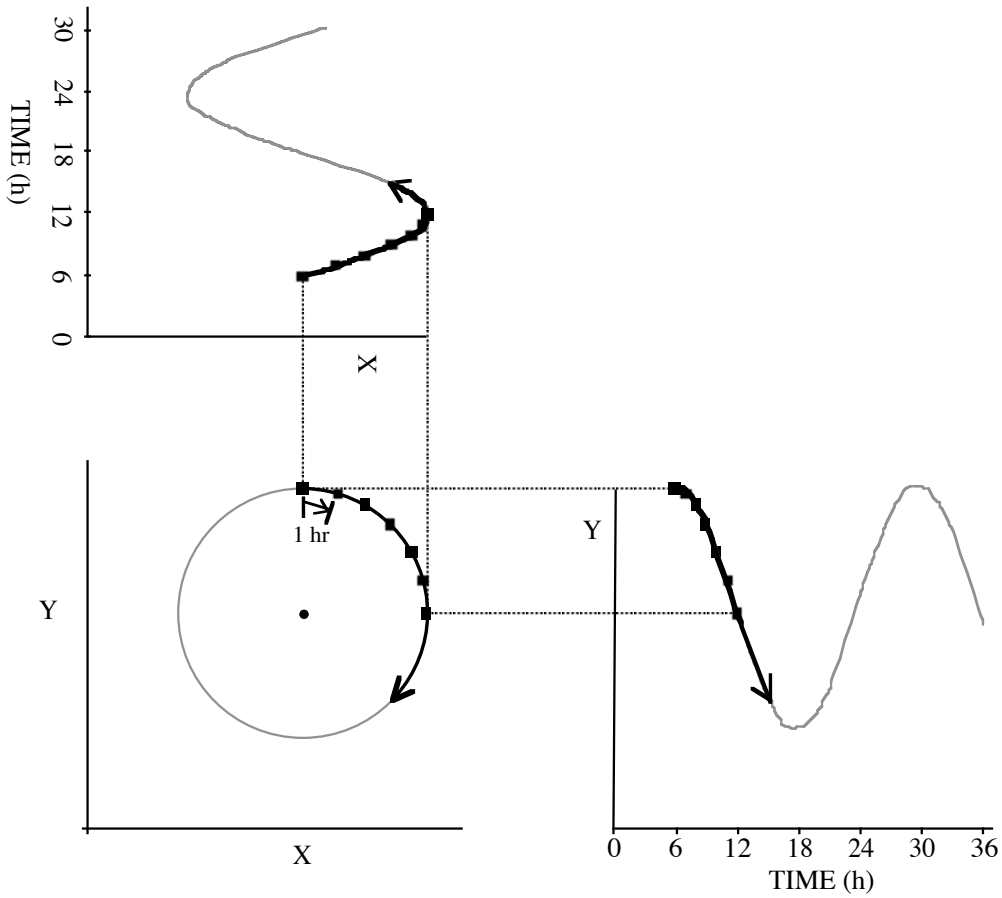
Since limit cycles are often misunderstood, I would first like to present some basics relative to the model that will be used. Following the basics, the role of the treatment as proposed by Peterson will be addressed; and finally the consequences of the displacement limit cycle model will be considered.

THE BASICS:

Phase Plane Plotting:

Although limit cycles can be explained by the conventional method of plotting the variable versus time, it is easier to visualize the features of a limit cycle in the form of a phase plane plot (Minorsky, 1962; Pavlidis, 1973) involving two interacting parameters. One parameter will be referred to as X and the other parameter as Y. The cellular nature of these parameters to date is unknown and could be just about anything such as interaction of adenylate cyclase activity (X) and Mg^{++} concentration changes (Y); mitochondrial shape (X) and glucose concentration (Y); transcription rates (X) and pH levels (Y); citrate concentration (X) and phosphofructokinase activity (Y); etc. In a **phase plane plot**, as shown in Figure 1, one oscillating parameter is plotted on one axis and the other oscillating parameter is plotted on the other axis. As time progresses, these parameters change value, thus leading to a trace on the graph over time. If the parameters repeat themselves over time then a closed loop will be formed; therefore a closed loop trace on a phase plane plot signifies a **sustained oscillatory phenomenon**. A phase plane plot of a perfect circle is represented by the two oscillating parameters each forming a perfect sinusoidal pattern with time, but 90 degrees out of phase with each other.

FIGURE 1. Phase plane plot of two interacting parameters, X and Y (lower left) and conventional plots of Y versus time (right) and X versus time (top). The plots of X versus time and Y versus time are sine plots 1/4 of a cycle different in phase.



The Poincaré Limit Cycle Equation:

There are many possible mathematical formulations that can describe a limit cycle (Pavlidis, 1973; Winfree, 1980; Winfree, 1986). Those equations that are represented by the X and Y Cartesian coordinate system are convenient in that they can be more easily related to feasible biological kinetics, but the mathematics are usually quite cumbersome. On the other hand, equations representing a limit cycle in polar coordinates (Θ and R) can be quite simple (Figure 2). The set of two equations used to represent the limit cycles presented in this paper have been referred to as the **Poincaré oscillator** (Glass and Mackey, 1988;Wanzhen et. al., 1992).

The first equation states that the rate of angular change is constant:

$$1) \quad \frac{d\Theta}{dt} = 360 \text{ degrees per } \tau \text{ hours} = \frac{2\pi}{\tau}$$

where: Θ is the angle (as defined by the way an analog clock moves)(a measure of phase);
t is time
τ is the natural free running period length of the rhythm.
π is pi=3.1416.....

The second equation is the essence of the limit cycle and describes how R will change over time:

2) $\frac{dR}{dt} = \varepsilon R \left(R_\infty - R\right)$

where: R is the distance from the singularity at any given time as dictated by the position of the X and Y parameters (unitless).
R_∞ is a positive constant representing the natural radius of the limit cycle. (R would become R_∞ as time approaches infinity.)
ε is a positive constant representing how fast the system returns to the limit cycle when perturbed (ε reflects transient effects).

When integrated the equations become:

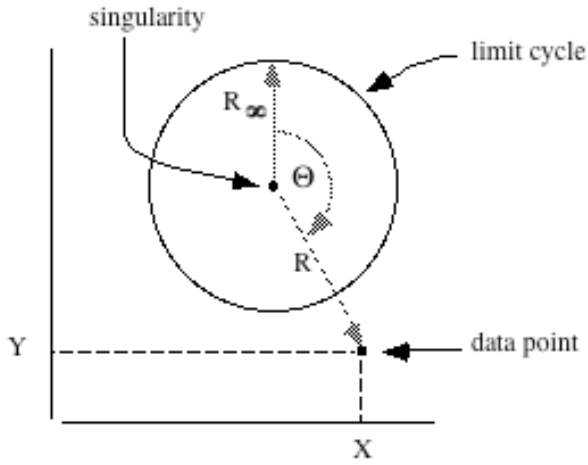
1i) $\Theta = \Theta_o + \frac{2\pi}{\tau} t$

where: Θ_o is the initial angle at time 0.

2i) $R = \frac{R_\infty}{\left(\frac{R_\infty}{R_o} - 1\right) * e^{(-\varepsilon * R_\infty * t)} + 1}$

where: R_o is the initial R at time 0.

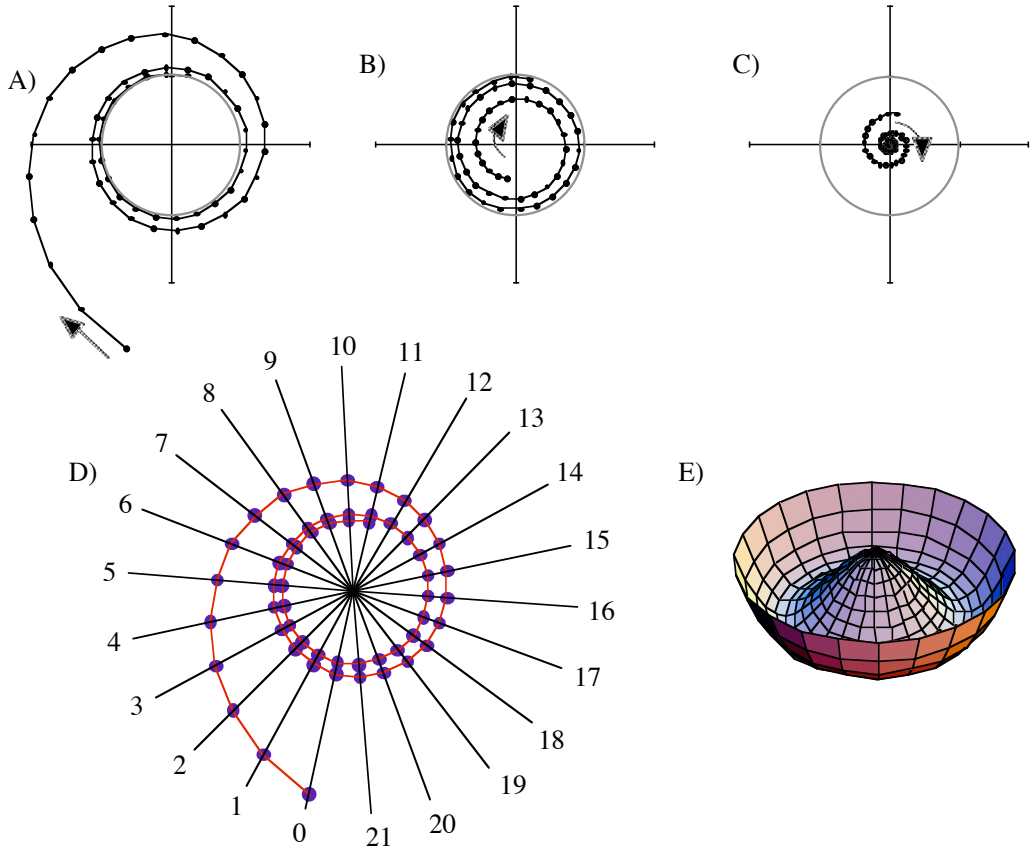
FIGURE 2. Diagram demonstrating Θ, R, R_∞, and the singularity as used in this manuscript.



Limit Cycle Behavior:

Note from equation 2) and Figure 3A) that if X and Y are in a position such that $R > R_\infty$ then rate chnage of the radius ($\frac{dR}{dt}$) will be negative, therefore R will decrease over time (Figure 3A). On the other hand, if $R<R_\infty$ (but $R \neq 0$) then $\frac{dR}{dt}$ will be positive and thus R will increase over time (Figure 3B). However, once $R=R_\infty$, then the R will no longer change ($\frac{dR}{dt} = 0$) (Figure 3A and 3B) and the limit cycle will have been

FIGURE 3. Limit cycle behavior using equations 1i) and 2i). Values used for these figures are: Θ_o(initial angle) = 3.2 radians, τ (free running period length) = 22 hours, ε (transient effect) = 0.06 and R_∞ (radius) =1 (indicated by shaded circle). For A) and D) R_o (initial R) = 3, for B) R_o = 0.5, and for C) R_o = 0.03. The oscillation proceeds in a clockwise fashion and time is represented by plotting a point for each simulated lapsed hour. Fifty five hours (2.5 cycles) of simulated data are shown. D): A replot of A) showing the isochrons (data points are larger for emphasis). E): A three dimensional phase plane plot where the third axis is the rate of change of dR/dt. The state variables would have to be balanced perfectly on the singularity to have no change occur; in all other cases the state variables would seek the trough of the bowl.



reached. The system will perpetually oscillate in the same cycle with the same wave form, amplitude and period.

A representation of **transients** is the behavior that occurs before the limit cycle is reached. The rate at which the limit cycle is reached is represented by the ε value and thus ε is a measure of the transient effect. Also note that the rate at which R changes gets smaller as R gets closer to R_∞, (because R_∞ –R gets smaller), thus the limit cycle is never precisely reached, it is only asymptotically approached (Figure 3A and 3B).

The **singularity** is represented when R = 0. If R =0 then the R should not change (dR/dt = 0), but if the X and Y parameters are such that R is minutely different from 0, then $\frac{dR}{dt} > 0$ and R will start increasing until the limit cycle is reached (Figure 3C, 3E).

The nature of cyclic phenomena requires that a system will pass through a particular phase each cycle. In a limit cycle a particular phase could be defined to be equal to the phase that is achieved exactly one period length later. Figure 3 was generated using a free running period length of 22 hours, therefore every 22 hours the system should return to an equal phase. By arbitrarily defining any point on the cycle as a particular phase (for example, use the phase point at time 0 in Figure 3D), then every 22 hours later that same phase should be repeated. In a limit cycle model this will create a family of points. One can draw a line, called an **isochron**, through this family

of points and define the isochron line as a particular phase (radial lines of Figure 3D). A characteristic of a limit cycle is that any time the system has X and Y fall upon an isochron (either through manipulation or natural progression), then the system will have the phase of that isochron at that time. The isochrons for the limit cycle presented here are linear, but for other limit cycle models it is common to have highly contorted isochrons (Pavlidis, 1973; Winfree, 1980). Another way to think about isochrons is that it if one simultaneously has several replicate systems with the only difference being that they are started at different points on the same given isochron, then after a sufficient time such that all the systems have reached the limit cycle, all the systems will exactly coincide.

MODELING THE TREATMENT INFLUENCE BY DISPLACEMENT OF THE LIMIT CYCLE:

Under a control set of conditions, a particular limit cycle will be in effect (for *Neurospora*, typically these control conditions are darkness and 25°C) as dictated by the effects of the environmental conditions upon the biochemistry and biophysics of the system. The essence of the displacement limit cycle model is that when different environmental conditions are applied (such as a different light intensity, temperature, or chemical concentration) then the biochemical and biophysical reaction kinetics change such that a new limit cycle comes into effect. The new kinetics take instant effect, although the physical oscillating parameters themselves will not instantly change. (An analogous example is a chemical reaction in a test tube. A sudden increase in solution temperature does not instantaneously change the concentration of the reactants, but it does instantaneously change the rate at which the reactants will subsequently react).

The treatment could influence the limit cycle in many ways. However, initial studies with the model indicate that the repositioning of the limit cycle accounts for most of the qualitative effects. Singling out the displacement of the limit cycle and simplifying, or keeping constant, all other factors as a function of treatment intensity is obviously not accurate (see 'Discussion'). Nevertheless, for simplicity and purposes of this paper, the radius of the limit cycle (R_∞) and the period length (τ) will be maintained constant for all conditions; and the only effect that will be considered is a displacement in a two-dimensional plane. Figure 4 shows these proposed effects for a stronger and stronger treatment influence. Figure 5 shows the geometry, trigonometry, and symbols used.

FIGURE 4. The displacement limit cycle model. A)-C): The stronger the intensity (Z) the farther the treatment limit cycle is displaced from the control limit cycle. Z is used for "zeitgebering effect". D): A specific example shows the dark limit cycle when the organism is in the dark and a light limit cycle when the organism is in the light of a particular intensity. A threshold effect is also modeled such that for values of Y above the threshold one form of physiology occurs while another form of physiology occurs when Y is below the threshold effect.

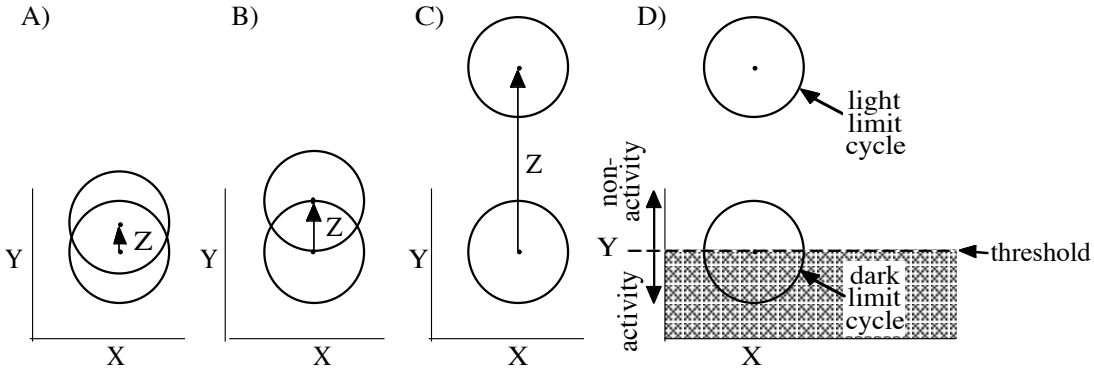


FIGURE 5. A) The relationship of the control limit cycle (which is typically dark and 25°C for *Neurospora*) to a limit cycle under the influence of a treatment (such as light or temperature that is different from the control). By definition:

3) $a = RZ\cos(\Theta Z)$

4) $b = RZ\sin(\Theta Z)$

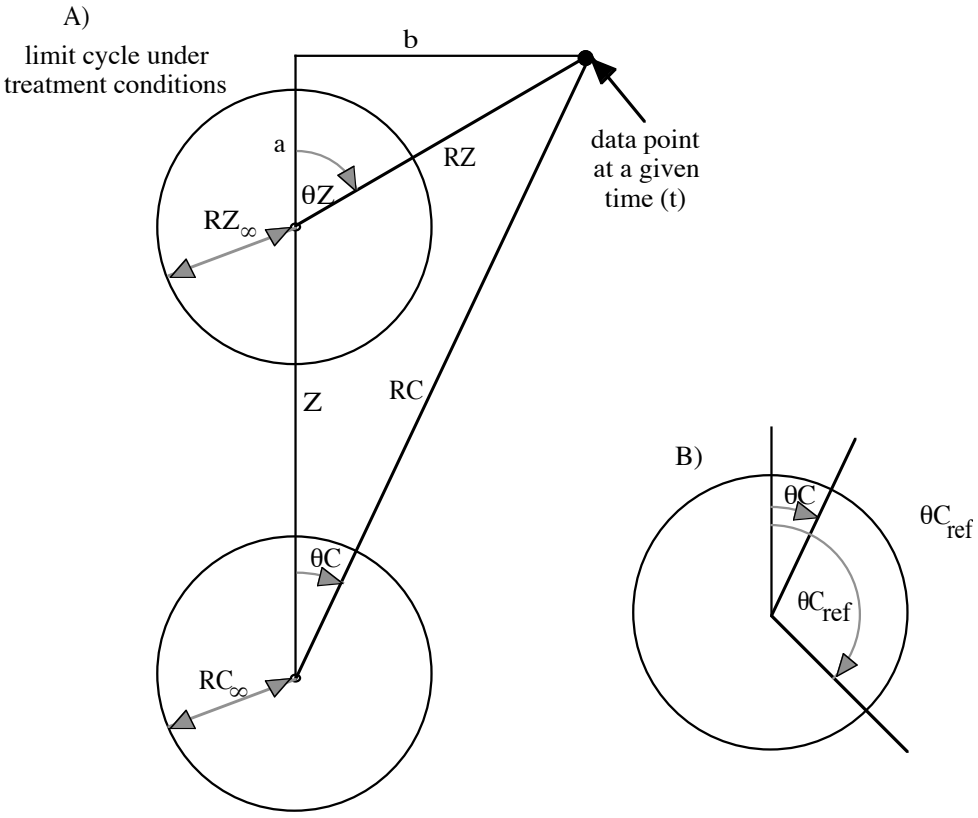
5) $\Theta C = \text{atan}\left(\frac{b}{a+Z}\right)$

Eliminate a and b from 5) by substituting in 3) and 4). Divide the numerator and denominator by the always positive RZ.

6) $\Theta C = \text{atan}\left(\frac{\sin(\Theta Z)}{\cos(\Theta Z)+\frac{Z}{RZ}}\right)$

B) A phase reference point under control conditions is often used by an experimenter to monitor their circadian rhythm (e.g., the start of activity, peak of luminescence, peak of photosynthesis, or peak of conidiation). This particular phase reference point is unique and different for each organism and each physiology being measured. The phase reference point will be identified here as ΘC_{ref}. Considering equation 1i), the time it takes to get to that phase reference point starting from a particular phase of ΘC would be:

7) Time to phase reference point $= \frac{\tau}{2\pi}(\Theta C_{ref} - \Theta C)$



It is also important to note that the amount of displacement (Z) is almost certainly not linearly related to actual environmental intensities. Furthermore, there may well be limits of how far a limit cycle can be displaced by a treatment. For example, strong phase shifting by bright light is often not observed (e.g., rat and mouse) possibly because the highest light levels can only displace the limit cycle a fraction of the distance of that which is possible in certain lower organisms, such as the fungi, where bright light does cause strong phase resetting responses.

The direction of movement of the limit cycle relative to intensity is totally irrelevant, in part because this is purely a mathematical formulation (and technically the origin of the graph is at the singularity). If one does not like the upward motion, one can simply rotate the graph. However, one can experimentally determine the direction of movement caused by one treatment relative to the influence of any other treatment (Gooch, et al., 1992).

It is important to distinguish the "hands" of the clock versus the actual process of the clock. The modeling is designed to represent the basic underlying cellular mechanism of the clock; in turn this underlying oscillator only influences what is overtly observed by the experimenter. For example it has often been reported that bright light will stop the observed oscillation of a circadian clock, but this does not mean that the underlying oscillator has necessarily stopped. A threshold effect may account for such an observation (Figure 4D) (Wever, 1965). Whenever a certain parameter (Y) is above a certain threshold level, possibly an ion concentration, the observable activity will occur; below the threshold level the activity is absent. If, in constant dark, the limit cycle causes the system to pass repeatedly through this threshold level then an oscillation of the activity will be observed. On the other hand, if the entire limit cycle is moved above the threshold in constant bright light, then the overt observation will be activity at all times even though the actual internal clock continues to cycle.

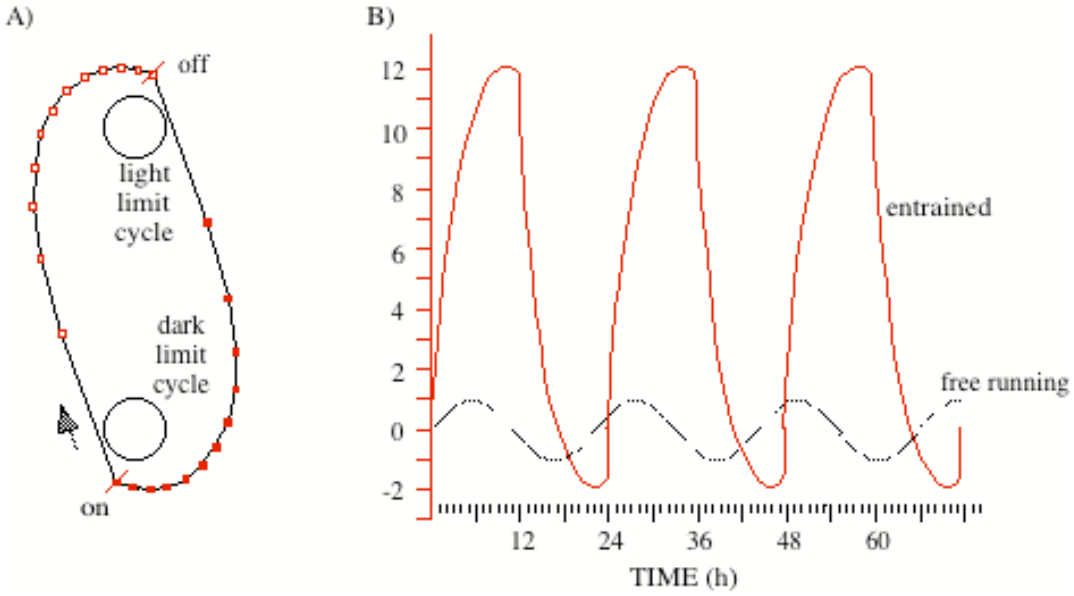
CONSEQUENCES OF THE DISPLACEMENT LIMIT CYCLE MODEL:

Entrainment:

When the light limit cycle is in effect, the parameters will seek the light limit cycle and begin to approach it and when the lights are turned off the parameters will then seek the dark limit cycle. If there is a regular cycle of simulated lights going on off (e.g. 12 hrs on, 12 hrs off) then for the first few cycles, the parameters undergo unusual fluctuations (which could probably be correlated with jet lag). But eventually, the parameters will fall into a unique entrained pattern around the two limit cycles (Peterson, 1980a). Shown in Figure 6 is the result of a computer simulation of 12 hours light-12 hours dark (12L:12D) using the displacement limit cycle model. The rapidity that the rhythm reaches the unique entrained pattern depends upon the transient value (the larger ε the fewer the transients). For Figure 6, the free running limit cycles were set up to be 22 hours, but the entrained cycle is exactly 24 hours, as expected. Note that the amplitude in the entrained cycle is much enhanced over the free running amplitude, a phenomenon that seems to be consistent with the fact that circadian rhythms are usually more robust under entraining conditions. Also note that the wave form of the entrained cycle shows more abrupt changes in shape compared to the more smooth flowing free running rhythms (the equations presented here create a perfect sine wave under free running conditions). When the free running period lengths are made to be exactly 24 hours, then a 12L:12D entrainment cycle will give the result that the circadian time 0 (CT0: the time of dark to light transition) and 180° phases of the limit cycle will coincide exactly. When the free running period lengths are shorter than the entrained cycle, then the CT0 falls to values slightly greater than the 180° phase angle of the limit cycles. When the free running period lengths are longer than the entrained cycle, then the CT0 falls to values less than the 180°. This creates different phase angle differences depending upon the light to dark ratio of the entraining cycle which can be related to previously seen experimental data (Aschoff, 1965; Wever, 1965)

The computerized simulated system will reach limits to which it will no longer entrain for either high or low entraining periods. There seems to be no distinct point at which entrainment is lost, but as these areas are approached the simulated oscillations go through seemingly erratic, yet structured patterns. It is not clear if this unusual phenomenon mimics actual data; this is an area under current investigation.

FIGURE 6. An entrained cycle of the displacement limit cycle model using a simulated 12 hours light:12 hours dark cycle where the light was simulated by a value of Z = 10 and dark with a value of Z = 0. τ (free running period) value of 22 hours and $\varepsilon = 0.06$ was assigned to both the dark and light limit cycles. A): The oscillation proceeds in a clockwise fashion and time is represented by plotting a point for each simulated lapsed hour. "ON" is the time in the cycle when the lights go on (ΘC_{on} = 3.47 radians, RC_{on} = 1.803, ΘZ_{on} = 3.19 radians, RZ_{on} = 11.717) and "OFF" is the time the lights go off (ΘC_{off} = 0.05 radians, RC_{off} = 11.717, ΘZ_{off} = 0.33 radians, RZ_{off} = 1.803). The open squares indicate data created in "on" conditions and the filled squares indicate data produced in the "off" condition. B) A plot of Y as a function of time for the entrained cycle as compared to the dark free running cycle.

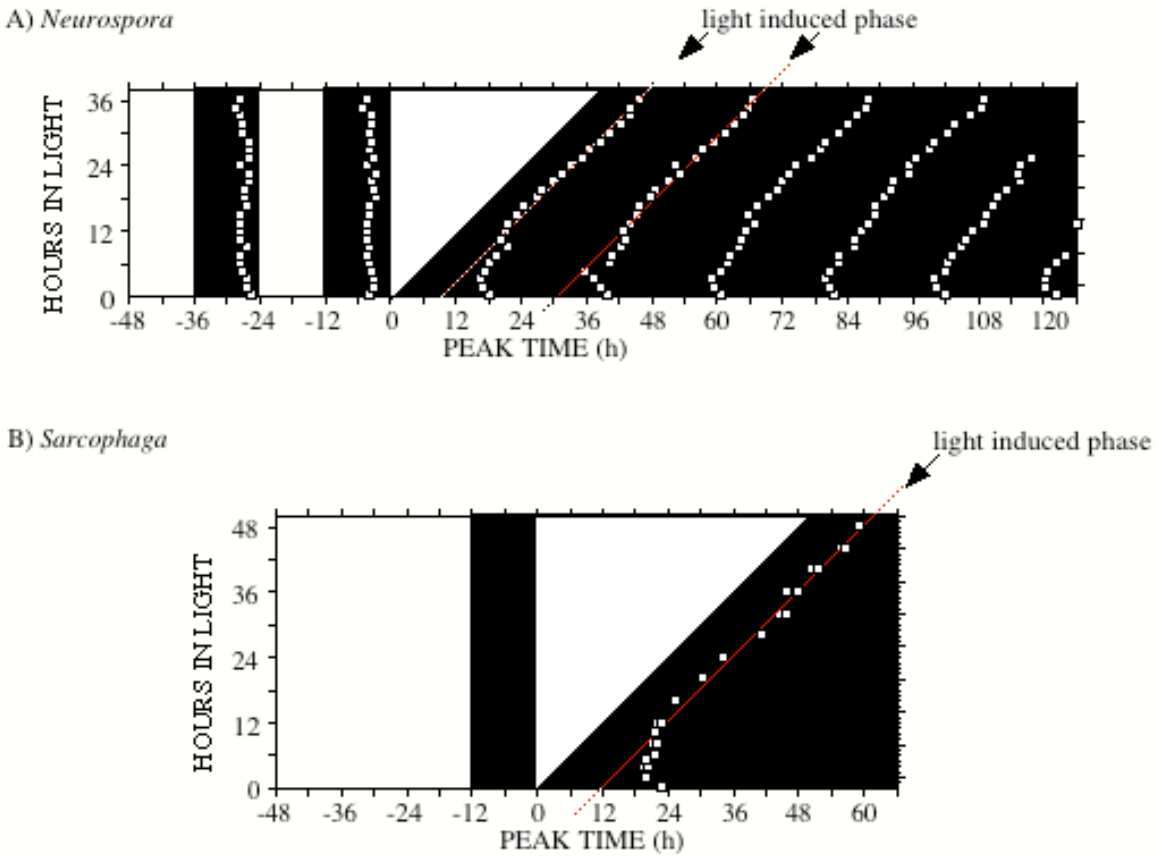


Induced-Phase Caused by Release-Assay Experiments:

It has been reported (Pittendrigh, 1960; Pittendrigh, 1966; Jones, 1976; Saunders, 1976; Gooch, 1985) in release-assay experiments using peak time response plots (Figure 7) that when organisms are put into light for a sufficiently long time, and then returned to darkness to measure the free running rhythm, that the resulting phase (light **induced phase**) is solely dependent upon the time at which the organisms are transferred from light to dark. That is, the light tends to cause the system to go to a particular phase (with respect to a rhythm subsequently determined in the dark) and the system stays at this light induced phase until the lights are turned off. One interpretation is that the clock simply goes to a certain phase point and stops, which will be termed the "holding hypothesis". However, this holding hypothesis does not explain why, for exposures of less than 12 hours in a release-assay experiment, phase advances are often observed relative to the control that received no treatment (Pittendrigh, 1974; Saunders, 1976; Gooch, 1985). Such phase advances can be seen in Figure 7 when one compares the data for 6 hours in light or less to the data that received 0.0 hours in light. It has been shown that the degree to which one gets these phase advances depends upon the pre-entrainment treatment (Pittendrigh, 1974; Peterson and Saunders, 1980; Gooch et al. 1992). Also, the holding hypothesis does not explain why, for longer light exposures in release-assay experiments, there often appears to be a slight oscillation ("wiggle") about the light induced phase line (Peterson and Saunders, 1980; Gooch et al. 1992). A hint of this wiggle can be seen in the data of Figure 7. It has been shown that this oscillation can be enhanced with lower intensities, and the period length of this oscillation is circadian in nature (Peterson, 1980a; Gooch et al. 1992).

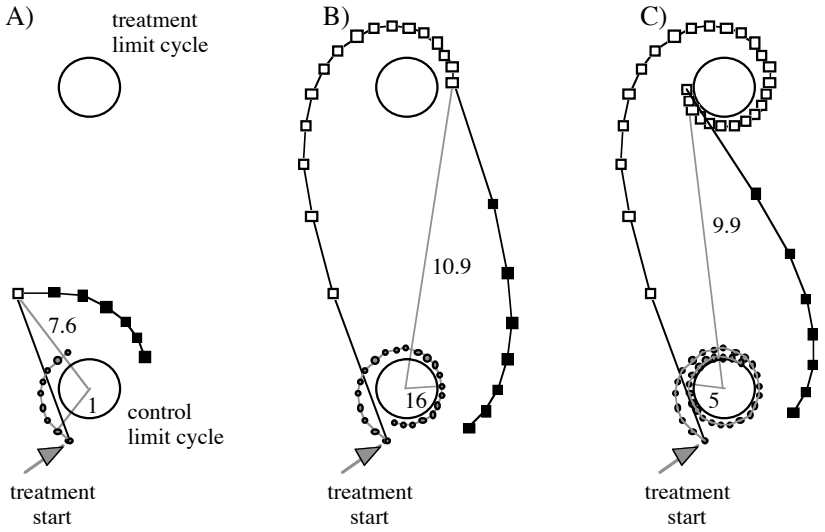
FIGURE 7. Peak time response plots for release-assay experiments where darkened areas represent dark treatment and nonshaded areas are light treatment. Data points indicate the peak time of the measurement. A): *Neurospora crassa* conidiation rhythm data (Gooch, 1965) 25°C, 4000 lux (~28 μE·m^{-2}·sec^{-1}). B): Replotted pupal eclosion rhythm data of *Sarcophaga* (Saunders, 1976) ~25°C, 240 μW cm^{-2} (~11 μE·m^{-2}·sec^{-1}).

In a release-assay experiment, all organisms are initially given the same treatment to get them all in equal phase. In the case of the *Neurospora*, all the cultures were treated with 12 hours light and 12 hours dark entrainment for two days, and then at the end of the last dark exposure different treatments were started for the different cultures. One culture (the control) remained in the dark and thus received 0.0 hours of light. The time at which there was maximal conidiation was determined for each day, and those peak times of conidiation are plotted along the x-axis. Thus, the bottom row of data points on the graph represent control organisms that received 0.0 hours of light. The next row of data points represents the conidiation peaks for another culture of *Neurospora* that received 1.5 hours of light exposure. The next row of data points represents a culture that received 3 hours of light exposure, etc.



Peterson and Saunders were intrigued by these anomalies relative to the holding hypothesis and developed the concepts (Peterson and Saunders, 1980; Peterson, 1980a) of what is being termed in this report as the displacement limit cycle model. Rather than assume that the circadian clock is being stopped by light, they entertained the idea that the light causes a new light limit cycle relative to the limit cycle that occurs in the dark (Pavlidis, 1973). Figure 8 shows how a release-assay experiment might progress with this model in mind. The simulated data points were generated using the equations 1i) and 2i). For the sake of discussion, assume the treatment is light and the control condition is darkness. The starting point was determined as the end of dark phase of a simulated pre-entrainment treatment of 12 hours light and 12 hours dark. A simulated organism

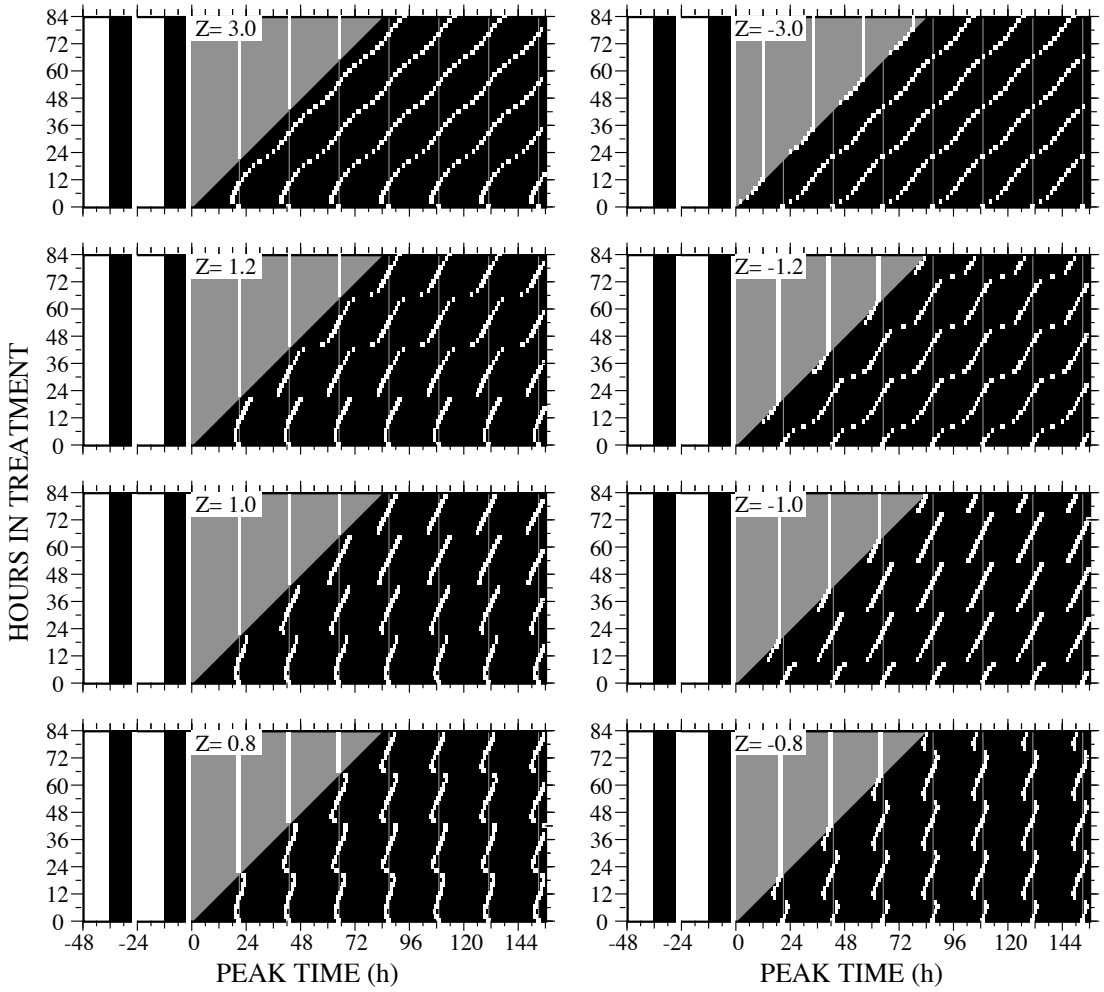
FIGURE 8. Computer generated displacement limit cycle model representation of a release-assay experiment showing three different times of hours in treatment. All equation constants are the same as that in Figure 6. The starting time of treatment is the same in each case and is the phase representing the beginning time of treatment if the system were entrained to a 12 hour control: 12 hour treatment entrainment cycle (CT = 0). A): One hour in treatment conditions (e.g., light). The open square represents the position of the parameters after a one hour exposure to the attracting treatment limit cycle. The shaded line to the open square represents the isochron that is generated due to that one hour treatment and the number (7.6 hours) next to the shaded line is the value for that isochron relative to the control limit cycle. After one hour of simulated treatment, the system is then returned to control conditions (e.g., dark) and the subsequent 7 hours under these conditions is represented by the darkened squares. For comparison an untreated control (e.g., constant dark) is shown for 8 hours by the shaded circles and the isochron of the control after 1.0 hours. B) 16 hours of treatment C) 27 hours of treatment.



1 hour treatment 16 hours treatment 27 hours treatment

exposed to one hour of light would have its clock parameters start to move quickly toward the new light limit cycle (Figure 8A). If, after the one hour the lights are turned off, the parameters would again move back toward the dark limit cycle. The organisms that received this treatment would be ahead (phase advanced) relative to the organisms that received no light treatment. Whether there is a phase advance depends upon a) where the system started relative to the dark limit cycle, which depends upon the pre-entrainment conditions, b) how long the organisms were exposed to the light, and c) the equation constants. If the simulated organisms are exposed to the light for long periods of time, the parameters will reach the light limit cycle and oscillate around it. The parameters would proceed to oscillate in that light limit cycle as long as the lights remained on. When the lights are turned off, the dark limit cycle again comes into effect. However, the parameters will be wherever the light limit cycle left them; if the light limit cycle was far from the dark limit cycle then the range of possible phases relative to the dark limit cycle would be small (the arc of possible isochrons would be small). Although the range of phases would be small, there should still be a range of phases dependent upon what time the system was transferred from the light to the dark (hence the wiggle). Therefore, this wiggle would be an oscillation about the light induced phase. Such an oscillation in data should represent the oscillation while it was in the light and the period length should correspond to the period length while in the light. Peterson plotted this oscillation about the light induced phase and determined a period length of about 24 hours (Peterson, 1980a). A complete release-assay experiment can be generated by the computer using a series of different light exposures; the simulated results of a release-assay experiment using the concepts presented in Figure 8 are shown in Figure 9 for different values of Z .

FIGURE 9. Simulated release-assay experiments using treatments of different intensities. For each panel there are two days of simulated entrainment as in Figure 6 of alternating 12 hours at Z = 10 (light areas) and 12 hours at Z = 0 (dark areas) (ΘC_{on} = 3.47 radians, RC_{on} = 1.803). This is followed by the treatment of various durations (shaded area) of indicated Z intensity followed by release back into the control conditions of Z = 0 (dark area on the right side of each plot). The left panels use positive values of Z and the right panels use negative values. The vertical dotted lines extrapolate upward the control data (Z = 0 during the treatment time). τ = 22 hours and ε = 0.06. Each point represents a simulated peak ($\Theta C_{ref} = \pi$ radians = 180°).

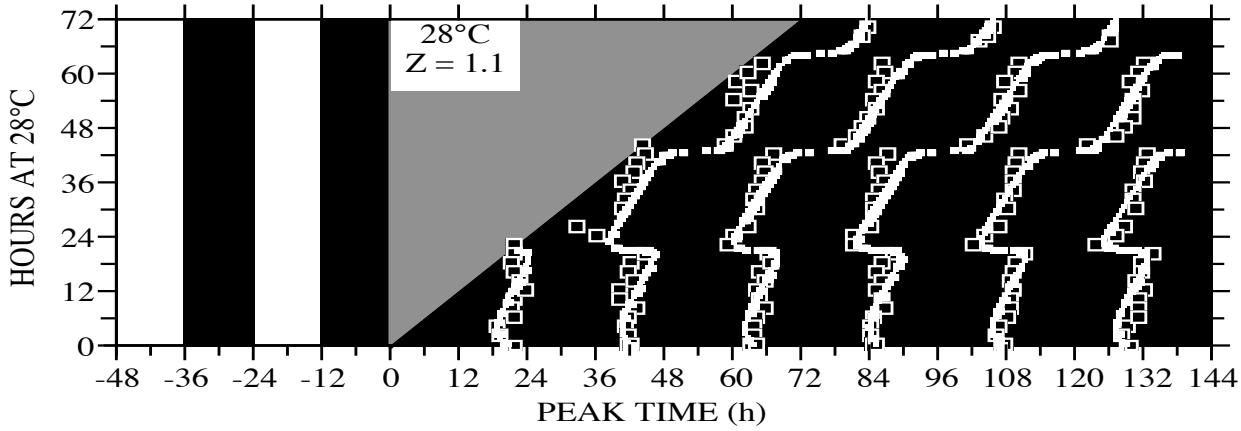


The model further suggests that if the same experiment is carried out with a lower intensity, then the amplitude of the wiggle should be enhanced. The computer simulation of this is shown in Figure 9. Peterson and Saunders (1980) carried out such experiments using the flesh fly, *Sarcophaga*, with light as a treatment and Gooch et al. (1992) used *Neurospora* with temperature as the treatment. The latter experiment was carried over a long enough time to see that there is indeed a repeatable oscillation about the temperature induced phase lines and that period lengths could be measured.

As seen in the modeling, an interesting phenomenon should occur when the intensity is low enough such that the treatment limit cycle passes through the singularity of the control limit cycle. The resulting data should show distinct discontinuities. Under these conditions, if the system is in the treatment limit cycle and the treatment is turned off just before it reaches the singularity point of the control limit cycle, then a phase close to the 90° isochron will be subsequently observed. However, if the treatment is left on just a moment longer so that the parameters pass to the other side of the singularity, then when the treatment is turned off the resulting phase should be close to the 270° isochron, therefore a sudden 180° discontinuous phase shift. The *Neurospora* data

(Gooch et al. 1992) that comes most close to showing this phenomenon is when 28°C was used as a treatment and 25°C was used as the control (the squares of Figure 10). The lines in Figure 10 were generated using equations 1) and 2) using constants indicated in the legend. The fit is obviously not precise, but the qualitative features do seem to be well represented.

FIGURE 10. A comparison of experimental data (boxes) of a release-assay experiment to a simulated release-assay experiment (points) using the displacement limit cycle model equations. The release-assay experiment was performed on *Neurospora* (Gooch et al. 1992). The mold was first inoculated onto race tubes and then they were exposed for two days to an entraining cycle of 12 hours light and 12 hours dark at 25°C. White areas represent light (~50 μE·m^{-2}·sec^{-1}) exposure at 25°C; black areas represent dark exposure at 25°C. At time 0, one set of race tubes (five race tubes per set) was maintained in the 25°C dark chamber, all other sets were put into a dark chamber at 28°C. After 2 hours of exposure, one set of race tubes was removed from the 28°C and placed into the 25°C dark chamber; after 4 hours another set was transferred, etc. At the completion of the experiment, the tubes were analyzed for the times at which conidiation peaked (peak time). Each data point (boxes) in the graphs represents the average peak time of conidiation for the five replicates.
The simulated data was obtained by first simulating two days of entrainment of alternating 12 hours at Z =10 (light areas) and 12 hours at Z = 0 (dark areas). This is followed by the treatment of Z = 1.1 followed by release back into the control conditions of Z = 0. The simulated peaks (points) were determined for every 0.2 hours of the vertical axis. The free running period that was used to make these plots was τ = 21.7 hours, this is the value that minimized the least squares. Other values used were ΘC_{on} = 3.47 radians, RC_{on} = 1.803, ε = 0.06, and ΘC_{ref} = π radians = 180°.



At even lower light intensities, the model shows new oscillations, but now the oscillation occurs about a vertical phase line (Figure 9: Z =0.8). Again, the experiments of Gooch et al. (1992) and Peterson (1980a) show this phenomenon. In fact, this changing phenomenon as a function of intensity is reminiscent of the change that occurs in phase response curves as one goes from type 1 to type 0 phase response curves (see discussion below regarding phase response curves).

Treatments with an intensity less than the control can also be used in a release-assay experiment. This is not possible if the treatment is light and the control is darkness; but it is possible if the treatment is a low light intensity relative to a brighter light control or if the treatment is temperature and one uses temperatures less than the control. The model suggests that a negative treatment should create a limit cycle in the opposite direction to that caused by the positive treatment. As a stronger and stronger negative treatment is used, all the same phenomena should be observed that were seen when increasing positive treatments were used. For example a strong negative treatment should create a treatment induced phase, with one difference being that it is about one half cycle out of phase of that created by the positive treatment. This phenomenon has again been observed using *Neurospora* and temperature (Gooch et al. 1992).

The simulated data of Figures 9 and 10 follow along unique curves depending upon the intensity of the treatment (Z) . The shape of these curves can be defined by an equation in closed form using equations 1i) and 2i) and the geometry defined in Figure 5. The time interval from the end of the treatment to phase reference point is defined by equation 7) of Figure 5:

$$8)\quad \mathrm{Time}_{\mathrm{end}\rightarrow\mathrm{ref}} = \frac{\tau}{2\pi}\left(\Theta C_{\mathrm{ref}} - \Theta C_{\mathrm{end}}\right)$$

The angle relative to the control limit cycle at the end of the treatment is defined by equation 6) and can be substituted into equation 8):

$$9)\quad \mathrm{Time}_{\mathrm{end}\rightarrow\mathrm{ref}} = \frac{\tau}{2\pi}\left(\Theta C_{\mathrm{ref}} - \operatorname{atan}\left(\frac{\sin(\Theta Z)}{\cos(\Theta Z)+\frac{Z}{RZ}}\right)\right)$$

ΘZ and RZ are defined by equations 1i) and 2i) and can be substituted into 9):

$$10)\quad = \frac{\tau}{2\pi} * \left(\Theta C_{\mathrm{ref}} - \operatorname{atan}\left(\frac{\sin\left(\Theta Z_o + \frac{2\pi}{\tau} D\right)}{\cos\left(\Theta Z_o + \frac{2\pi}{\tau} D\right) + Z * \left(\frac{1}{RZ_\infty} + \left(\frac{1}{RZ_o} - \frac{1}{RZ_\infty}\right) * e^{\left(-\varepsilon * RZ_\infty * D\right)}\right)}\right)\right)$$

where D is the time duration of the treatment.

In certain cases, it is easier to consider the initial conditions relative to the control limit cycle (ΘC_0 and RC_0) instead of the treatment limit cycle (ΘZ_0 and RZ_0). ΘZ_0 and RZ_0 can be replaced in equation 10) by using the following equations derived from the trigonometry of Figure 5:

$$11)\quad RZ_o = RC_o * \frac{\sin(\Theta C_o)}{\sin(\Theta Z_o)} \qquad 12)\quad \Theta Z_o = \operatorname{atan}\left(\frac{\sin(\Theta C_o)}{\cos(\Theta C_o) - \frac{Z}{RC_o}}\right)$$

Thus, the time to the peak from the end of the treatment is definable by the intensity (Z) , the time duration of the treatment (D), and the point in the cycle at which the treatment was initiated (ΘZ_0 and RZ_0). In a release-assay experiment ΘZ_0 and RZ_0 are kept constant (determined by the end of the pre-entrainment conditions), Z is kept constant, and the pulse duration time (D) is varied. The simulated data and curves of Figures 9 and 10 were generated using equation 10).

Phase Shifts Caused by Pulses:

One of the most commonly used experimental protocols in circadian rhythms is that which results in **phase response curves (PRCs).** A treatment is applied to a free running circadian system for a specified time and then the system is returned to control conditions (pulse) and the resulting phase is compared to a free running rhythm that had no pulse applied. A plot of the phase difference as a function of the time at which the pulse was applied is the phase response curve plot.

The displacement limit cycle model yields phase advances and phase delays depending upon when the pulse is given (Figure 11 demonstrates these characteristics). Without a treatment being applied, the parameters will simply cycle into the control limit cycle (small shaded circles of Figure 11A). However, when the treatment is applied for a duration of one hour, then the parameters will move rapidly toward the attracting treatment limit cycle (open box of Fig 11A). The farther the parameters are away from the influencing limit cycle, then the larger is the change per unit time, thus, accounting for the large jump in one hour. After one hour, the system is placed back under the control conditions and the control limit cycle again becomes the influencing limit cycle (filled boxes of Fig 11A). However, the large change in parameters that occurred while the treatment was active caused the parameters to jump well ahead in the cycle compared to the unperturbed control (causing a 6.6 hour advance). If the treatment is applied at another time in the cycle, the parameters can jump backwards relative to the control, and thus cause a phase delay (11.8 hour delay, Fig 11C). The amount of shift relative to the time the pulse is applied in the cycle is plotted in Figure 11E.

The situation also exists that if a pulse is given for the exact right duration starting at an exact particular phase, it could place the parameters directly onto the singularity (Figure 11D).

The phase response curve shown in Figure 11E shows a strong type 0 phase response curve, but the model can also show type 1 phase response curves (Peterson 1980a) when the intensity is reduced (e.g., Figure 12, Z = 2.5) or the duration is reduced (Figure 15, D = 0.1). Note that for Z = 2.5 of Figure 12 there are slight phase advances starting at circadian time = 0 (CT0) which progress into the phase delay region. Then, from about CT6 to CT18, there are the phase advances. These results are consistent with many observed phase response curves using light pulses (e.g., see Figure 14B). Similar curves have been seen using several other mathematical models of circadian rhythms.

In Figure 12, the effects of different treatment intensities are shown using the classic phase response curves (right panels) and the peak time response plots (left panels). The peak time response plot allows one to see easily the similarities in results and design of release-assay experiments compared to pulse experiments.

The effects of the transient term (ε) can be seen in the peak time response plots such as Figure 12, Z =3.4. As one moves up this plot, one can see a change in the pattern that is reminiscent of transient effects and this phenomenon does change relative to the transient term.

A negative treatment value would imply a treatment limit cycle in a direction opposite to a positive treatment limit cycle. In Figure 11, a negative treatment would be achieved if the limit cycle were below the control limit cycle rather than above it. In the simulated experiment (Figure 12, Z = -2.5), a negative treatment causes the phase response curve to be shifted one half cycle along the x-axis. Zatz et al. (1988) performed an interesting experiment that has the effect of creating a negative treatment using light. Using melatonin rhythms in chick embryo cells, he established a free running rhythm under control conditions using red light. Giving pulses of white light during the control conditions yields a classic type of phase response curve (Figure 13A). Using the same control conditions of red light, he also did pulse experiments using pulses of darkness (a negative treatment relative to the control), and he obtained a phase response curve shifted approximately one half cycle along the time axis (Figure 13A). The solid lines of Figure 13 are generated from the equations presented here (a type 0 phase response curve is shown, but the data was at such a transition point that a type 1 phase curve would have fitted almost as well). In panel A a positive Z value was used and in panel B a negative Z value was used.

FIGURE 11. Computer generated displacement limit cycle model representation of a phase response curve experiment showing four different times in the free running cycle at which a treatment pulse was applied. All initial equation constants are the same as that in Figure 6 and 8. The starting position (time = 0) in each case is the phase generated at the end of a pre-entrainment treatment of 12 hours treatment (e.g., light): 12 hours control (e.g., dark). A) A one hour pulse (e.g., light) given at time 0. The open square represents the position of the parameters after one hour of being attracted to the treatment limit cycle (e.g., light)(Z = 10). The simulated organism is then returned to control conditions (e.g., dark) and the subsequent 7 hours under these conditions is represented by the darkened squares. For comparison the untreated control (e.g., constant dark) is shown by the shaded circles. The relative positions of the isochrons of the treated and untreated systems are shown and the effect in this case was a 6.6 hour advance. B) A one hour pulse at time 7.095 yields a 0 hour phase shift. C) A one hour pulse at time 19 yields a phase delay of 11.8 hours. D) A 0.172 hour (10.3 minute) pulse at time 19.15 puts the system essentially onto the singularity (It was also determined from the model that if a reduced intensity of Z=3.4 is used, then a one hour pulse experiment starting at time 16.63 would also place the system essentially onto the singularity.) E. The phase response curve generated by applying one hour pulses (Z = 10) at different times in the cycle. The phase shifts indicated by diagrams A), B), and C) are indicated.

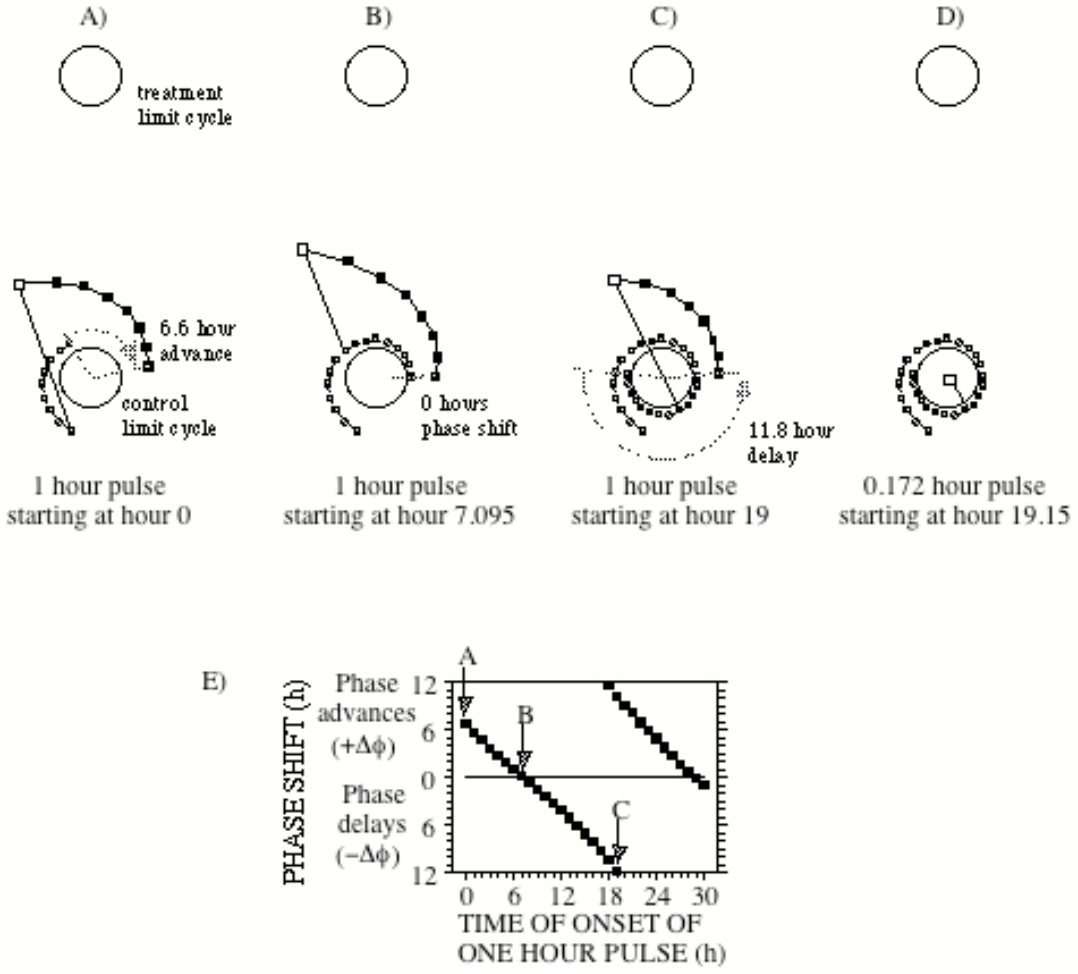


FIGURE 12. Computer generated displacement limit cycle model representation of the effects of a one hour pulse at four different treatments (Z = 5.0, 3.4, 2.5 and -2.5). All other initial equation constants are as indicated in Figure 6. The left panels plot the data as peak time response plots; the dark areas represent no treatment (i.e., Z = 0), the light areas during the first 48 hours of entrainment used a treatment Z = 10, and the light area representing the pulse uses a treatment of the indicated intensity. Note that the bottom panel uses a negative treatment as a pulse. The right panels are plotted in the format of phase response curves, which is commonly used for pulse data. The phase response curves are normalized to 24 hours (circadian time) on both axes. The x-axis of

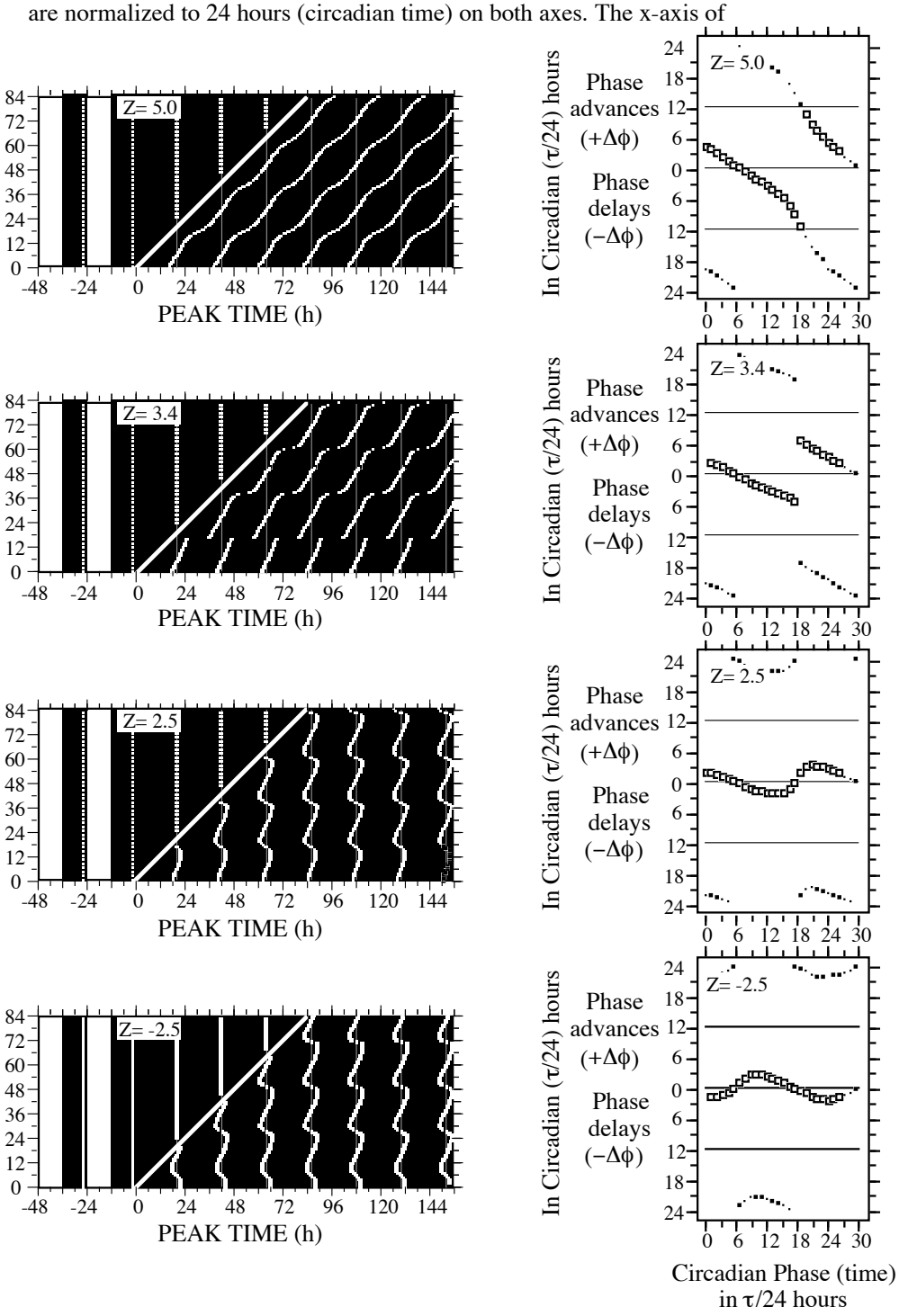
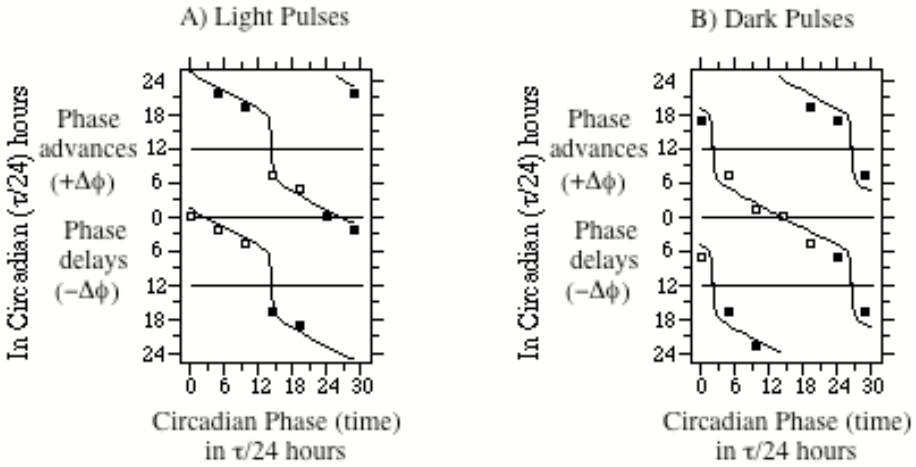


FIGURE 12. continued: circadian phase time represents the time of the pulse onset, and time 0 (CT0) is intended to represent the phase at which the treatment of Z = 10 would be expected to be applied in 12:12 entrained conditions (ΘC_{on} = 3.47 radians, RC_{on} = 1.803; see Figure 6). The open squares indicate the simulated data points that would conventionally be plotted in a phase response curve, the small filled squares represent other valid data points that help visualize the trends.

FIGURE 13. Phase response curves for 4 hour pulses of white light or darkness on melatonin production of chick pineal cells (Zatz et al., 1988). Except for when the pulses are applied, the cells are maintained in a constant red light. The open squares indicate the data points reported by Zatz et al. (1988) normalized to circadian time (using a free running period time of 20 hours) and the small filled squares represent how these data points would presumably repeat themselves under circadian conditions. The solid lines are generated by the equations presented in this report using the following initial values: ΘC_{on} = 3.47 radians, RC_{on} = 1.803, ε = 0.06, $Z_{\text{red light}}$ = 0, τ = 20, pulse duration 4 hours. The only difference in the two curves is that in A) the intensity (Z) was a positive value 1.6 and in B) it was a negative of -1.6.



Equation 10) describes the exact shape of the phase response curve for this model when duration (D) is kept constant and the time of pulse application (ΘC_0) would become a variable. When Z is varied from zero to very large values in pulse experiments using equation 10), there is a prediction that all the data should fall into a very limited range, Figure 14A. A similar prediction has been made using different models (Winfree, 1980; Guevara and Glass, 1982). To compare this prediction with experimental data, all the data from the PRC Atlas (Johnson, 1990) using pulses of light of two hours or less (ultraviolet and far red light data was excluded) was plotted in a phase response curve format and compared to the range predicted above. Despite the over simplicity of the displacement limit cycle model and despite the dangers of plotting the data from 97 experiments in the same format, it is interesting that most of the data does in fact fall within the expected range. It is also interesting that the one set of data that conspicuously falls out of the range (highlighted by open squares) is the unique and classic experiment by Bruce et al. (1960) using 0.5 millisecond strobe light to cause phase shifts in spore discharge of the mold *Pilobolus*. It is not clear why this data should be any different than the others.

FIGURE 14. A) Phase response curves of computer generated displacement limit cycle model representation of the effects of a one hour pulse at seven different intensities. All other equation constants are as indicated in Figure 12. B) Given the conditions in panel A), the shaded area represents the area in which phase response curve data should be limited for varying positive intensities. The dark squares represent all data from the PRC Atlas (Johnson, 1990) where two hours or less of light (excluding u.v. and far red) were used to generate a phase response curve. 1238 points are represented, 268 of which involve phase shifts of 5 hours or more. The data represented by the open squares is from Bruce et al. (1960). The PRC Atlas data that fell into the indicated category and was used is as follows: A-Cr-1, A-Eg-3, A-Pm-1, C-Nc-1, C-Nc-11->18, C-Ps-1, D-Aj-1, D-Kb-1->9, D-Lg-1, E-Ac-3, F-Ag-1, F-Ap-1, F-Cq-1->2, F-Da-1->10, F-Dm-2, F-Dp-1->15, F-Pg-1->3, F-Sa-1, G-Lp-1, G-Pb-1, H-Al-1, H-Dm-1, H-Gv-1->2, H-Ma-1->2, H-Ma-5->16, H-Ma-18, H-Ma-20->21, H-Mb-1, H-Mm-1->2, H-Pl-1, H-Pm-1, H-R-6, H-Rn-1->2, H-Ss-1->2, H-So-1->2, H-Ts-1, H-Th-1, H-Tm-1.

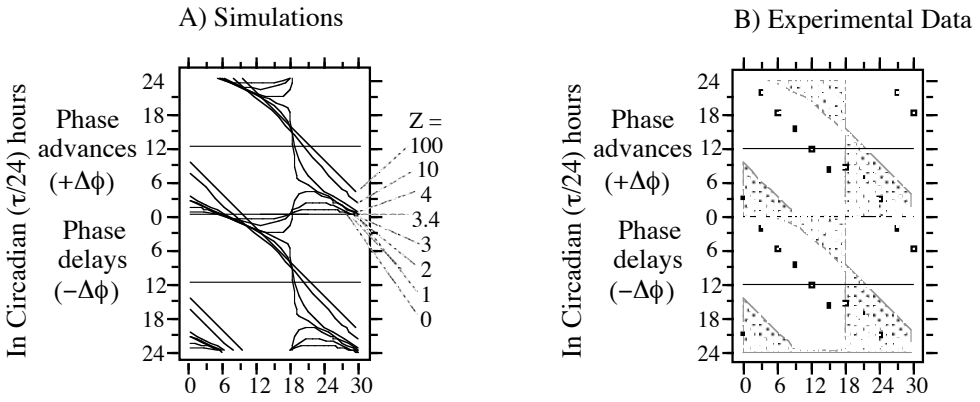


Figure 15 shows the changes that occur when the pulse intensity is kept constant (Z = 10), but different pulse durations are used. Again type 0 or type 1 phase response curves can be generated depending upon the pulse duration. The simulated data for D = 2 hours or more, shows that a large pulse duration can drive the system to a particular phase point relative to the control conditions (induced phase) much as what was seen in the release-assay experiments. Thus, for long durations the time to the peak from the end of the pulse is relatively constant, independent of the pulse duration. However, this only applies for relatively large treatment values (See Fig 16B and results below when Z = 0.7).

The x-axis of a phase response curve is essentially the time in a cycle when a pulse is applied. Since one goal of phase response curves is to compare data from different experimenters using different pulse lengths, then it must be asked is it best to use the start of the pulse, the middle of the pulse, or the end of the pulse as the '**time when the pulse is applied**'? For short pulse durations this is not a major problem, but for longer pulse durations (e.g., pulses of more than two hours) the problem becomes significant. The PRC Atlas (Johnson, 1990) suggests the use of the pulse onset as a standard, and that standard has been followed in this manuscript in all previous figures. Phase response curve plots of different pulse durations are overlaid in Figure 16 using the equations derived from the displacement limit cycle model. The left panels of Figure 16 shows how the different phase response curves compare when the pulse onset is used as the reference of the time of the pulse application. Ideally we would like to see these overlapping curves show a distinct pattern similar to those of Figure 14A when different intensities were used, but this is obviously not the case. For a strong treatment (Z = 10) the situation is dramatically improved when the time of the end of the pulse (offset) is used as the reference (Fig 16A, right panel). But for a smaller intensity (Figure 16B, Z = 0.7), using the offset appears to be no better than the onset. For a medium range intensity , the time of onset seems to work the best for short durations and the time of offset seems to work better for longer durations. This last conclusion from the displacement limit cycle model seems to be consistent with the actual results of Saunders (1978) when he used different pulse durations of light on the pupal eclosion rhythm of the flesh flies, *Sarcophaga*.

FIGURE 15. Computer generated displacement limit cycle model representation of the effects of four different durations (D) of pulses at a particular intensity (Z = 10). All other details are the same as Figure 12.

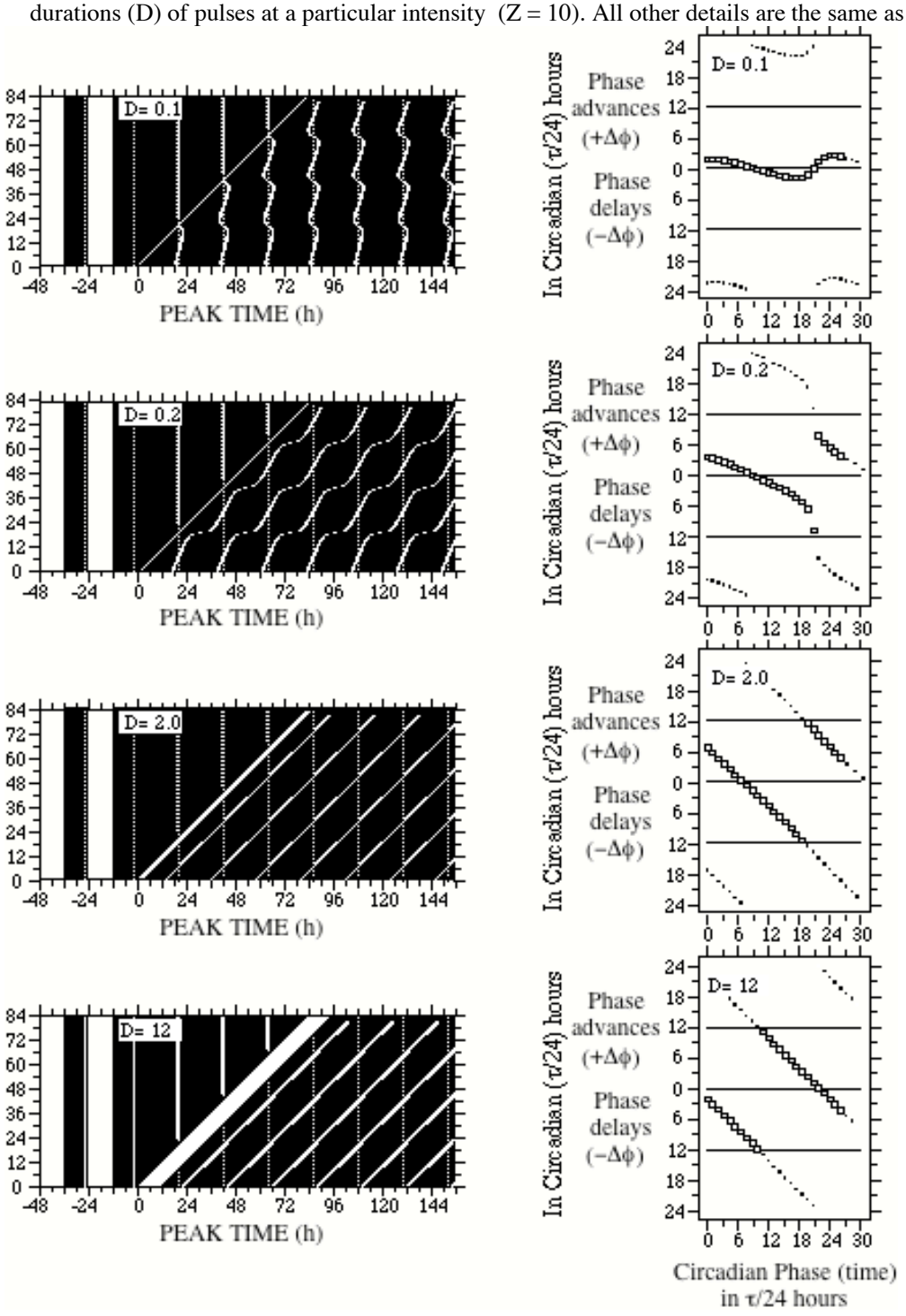
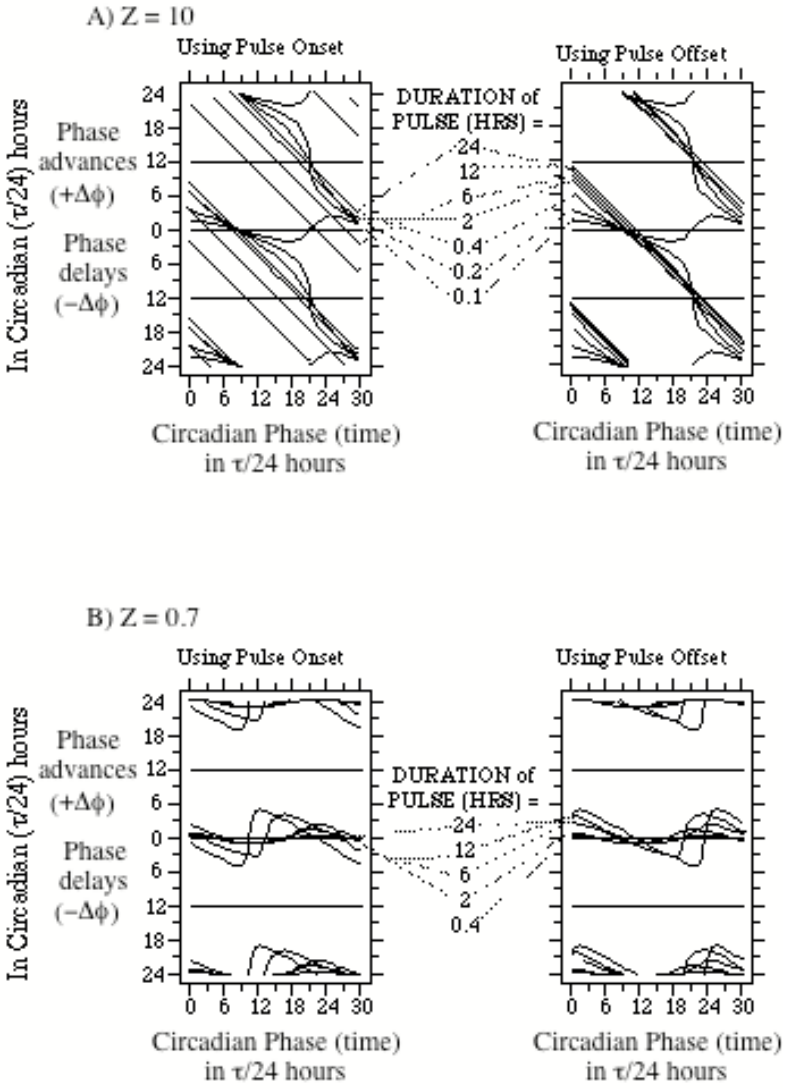


FIGURE 16. Phase response curves of computer generated displacement limit cycle model representation of the effects of different durations of pulse at a intensity of A) Z = 10 or B) Z = 0.7. In such phase response curves, the x-axis is the time in the circadian cycle that the pulse was applied. In the left panels this time of the pulse application was determined using the start (onset) of the pulse; in the right time of the pulse was determined using the end (offset) of the pulse.



The displacement limit cycle model, as presented, implies that light onset and light offset are of equal importance, and it mostly depends upon which most recently occurred. There seems to be some experimental support for this claim, for example the work with *Gonyaulax* (Figure 2 of ref. Gooch et al. 1992) suggesting that a dark induced phase can exist just as well as a light induced phase and the results of Zatz (Figure 13) showing dark pulses are as effective as light pulses.

Discussion:

Many of the qualitative features of several experimental protocols (such as entrainment, phase release, and phase response curve experiments) seem to be well mimicked by the displacement limit cycle model. Preliminary investigation suggests that these features are primarily dependent upon the trigonometry associated with the limit cycle being displaced by a new environmental condition (Figure 5), and only secondarily dependent upon the specific equations of the limit cycles. The displacement concept has been common to the modeling of Pavlidis (1973), Peterson (1980a), and Gooch (1992) resulting in similar conclusions, yet each of us has used somewhat different limit cycle equations.

Many models (e.g., Glass and Winfree, 1984; Glass and Mackey, 1988 and Zeng et. al., 1992) simply assume that a perturbation moves the parameters a specified distance on a phase plane plot in a specified direction. These models offer little reasoning as to what caused parameters to suddenly jump to a new position and it offers little understanding what would happen if they were left in the perturbed state. However, results of these **parameter shift models** display some similar features of the limit cycle displacement model presented here. These models often claim a **'fast returning' oscillator**; applying this special case of a large ε to the displacement limit cycle model would cause the parameters to be instantly attracted to a new limit cycle of equal size thus causing a jump in parameters a specified distance in a specified direction. Thus, for infinitely large ε the two models yield the same results. However, experimental evidence suggests that not all circadian oscillators are fast returning, and certainly none of them are infinitely fast returning. Even in *Neurospora*, which is thought to be relatively fast returning circadian rhythm, the parameter shift models can not easily account for certain experimental observations (such as those seen moving up the vertical axis of Figure 3) whereas the displacement limit cycle model can. Particularly interesting is the data that suggest as transients take place the system can go from one side of the singularity to the other (type 0 to type 1 transition). See Peterson (1980a) and Gooch (1992) for further discussion on this issue.

Although Czeisler et al. (1989) do not specifically use the displacement model, the assumption in that model that the time derivative of light acts upon the pacemaker could be consistent with the displacement limit cycle model. In the displacement limit cycle model the brighter the light the farther the light limit cycle is from the control dark limit cycle. The farther the light limit cycle is from the control dark limit cycle the faster the parameters will move toward that light limit cycle (equation 2) when shifted from dark to light.

Nevertheless, the subtle differences created by using different limit cycle formulations may be of value in determining what are the best equations to use. Preliminary investigations are being carried out using non perfectly circular limit cycles and nonperfectly radial isochrons in the displacement limit cycle model. In particular, the limit cycles that have been extensively examined by Pavlidis (1973) are being investigated. A variation of the Poincaré oscillator of equation 2) is also being explored:

The equation reduces to equation 2) when n = 1. When n = 2, the equations 1) and 2') are more easily translated into Cartesian coordinates (see equation 2 of ref. Peterson, 1980a). Also, when n = 2 and ε is small, the equations more closely resemble the van der Pohl equations (Wever, 1965; Minorsky, 1962; Peterson, 1980a). Kronauer and Czeisler (Kronauer, 1987, Czeisler et al., 1989) used the van der Pohl equation with high ε to derive a phase response curve equation and not surprisingly, that equation bears many similarities to equation 10) of this paper.

Some specific refinements of the model can be easily made to account for known effects. For example, for many systems it is well known that temperature does have a small predictable effect on the period length of the free running rhythm, and therefore τ could be made a function of temperature in equation 1). Similarly, it has been demonstrated that the free running period length is somewhat affected by light intensity and quality, and again the model could be modified such that τ reflects these effects. Such modifications would undoubtedly have to be made to be species specific.

The treatment intensity (Z) used in the displacement limit cycle model is a relative term. For example, higher light intensities may not always mean an increasing Z value if the organism has no way of detecting the brighter light or has no way of transmitting the information to the circadian oscillator. Indeed, some organisms

seem not to demonstrate strong phase resetting even with very intense treatments. Thus, the actual relationship of Z to intensity is not necessarily linear and would be species specific and treatment specific. Kronauer (1987) and Peterson (1980a) have proposed specific formulations of how Z should relate to actual light intensities.

The transient term (ε) in the Poincaré oscillator reflects how fast the oscillating parameters asymptotically return to the limit cycle (a larger ε means a faster return). The value of ε = 0.06 used throughout this manuscript seems to represent *Neurospora* data fairly well. The influence of the value of ε is most easily seen in looking at the data vertically in peak time response curves (e.g., Figs. 9, 10 and 12). The theoretical data stabilizes rather quickly, within a cycle or less, reflecting that ε = 0.06 is a relatively large value and that *Neurospora* is a quick responder to changes. The term $1/(\varepsilon * R\infty)$ has units of hours and would have a value of 16.7 hours when ε = 0.06 and $R\infty = 1$. Given these values and equation 2i), it takes 1.6 hours for R to decrease half way from 10 to 5.5, 6.8 hours to go from 2 to 1.5 and 10.1 hours to go from 1.2 to 1.1.

Pittendrigh and others (Bruce et al., 1960; Pittendrigh and Daan, 1976) have often observed changes in data along the x-axis of peak time response curves. These **'after effects'** are often attributed to coupling between different oscillators or lag time coupling between the oscillator and the overtly observed physiology. As presented, the displacement limit cycle model is unable to demonstrate any 'after effects', however there would be some influence along the x-axis using the model if the free running period lengths were different for the control limit cycle and the treatment limit cycle.

With respect to entrainment, the displacement limit cycle model implies that the entrained cycles should have a significantly larger amplitude and involve more abrupt changes compared to free running conditions. One consequence, if this conclusion is true, is that the entrained and free running rhythms are not simply a difference in scale as is often implied in defining 'circadian time'. For example, the peaks and troughs of an entrained wave form would not necessarily be at the same phase point as those of a free running wave form.

Future investigations of the displacement limit cycle model will be made to see how well it mimics other experimental protocols such as limits of entrainment, gradual turning on and off treatments corresponding to sunrise and sunset, phase relationships using different L:D entrainment regimes, skeleton photoperiods, and double pulse experiments. The goal of this modeling is not only to see how well the model fits published data but also to suggest new experimental protocols that may tell us more about how changes in environmental conditions affect circadian rhythms.

It must be kept in mind that the displacement limit cycle model is just a model. In fact, it is disconcerting that many of its features were selected on the basis of simplicity. Even though the model seems to mimic the data well, it seems to offer no obvious clues to the cellular and biochemical basis of the mechanism that causes the rhythm. The model was simply built from oscillator theory rather than using possible cellular kinetics as a starting point. Furthermore, the model does not account for temperature compensation, this feature is simply built into the model.

Since various types of experimental data can be qualitatively mimicked using the displacement limit cycle model, this then implies that entrainment, induced phase, phase advances, phase delays, etc., may well be part of the same phenomenon. For example, it seems to be useful to think that an entrainment experiment is simply a form of a pulse experiment, step experiment, or release-assay experiment. Unfortunately, experimenters often plot each type of experiment in very different ways, thus, often hiding the similarities between their results. Of course it is important for experimenters, as they alter the environmental conditions of their circadian systems, to look for unique features that their results present; but, it is equally important to seek and find the similarities.

SOFTWARE AVAILABILITY: Software that dynamically demonstrates the displacement limit cycle model can be requested (designed to work on a Macintosh).

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