Isochron-Based Phase Sensitivity Analysis of Oscillatory Biological Systems

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Abstract

Circadian rhythms possess the ability to entrain their internal phase to that of the environment. This ability relies on the phase response of circadian gene regulation to different environmental cues, of which light is the most obvious and important. Analyzing phase response of oscillatory systems requires the development of methods specific for these systems' attributes, such as the period and phase. This article presents two phase analyses of oscillatory systems for infinitesimal and finite perturbations, based on the isochron as a phase measure. In addition, phase response curves can be computed from the results of the analyses. Finally, the application to investigate the phase behavior of *Drosophila* circadian rhythm gives experimentally testable predictions for the control mechanisms of circadian phase and period responses.

1 Introduction

Circadian rhythms regulate daily activity cycles of many different species, from the unimolecular Neurospora to highly multicellular mammals, as an evolutionary adaptation to the earth's rotation. The rhythm is governed by transcriptional-translational-posttranslational regulation of several key genes which produces endogenous oscillations of the mRNA and protein level with a period of approximately 24 hour (hence the term *circadian* meaning about a day). Although the genes differ from species to species, the architecture of circadian gene network is remarkably preserved across different species suggesting an evolutionary convergence [1, 2]. The effectors of circadian rhythm control many hormonal, physiological, and psychomotor performance functions, which among other things give the overt rest-activity cycle [3]. The core of this rhythm consists of multiple feedback loops (coupled negative and positive feedbacks) giving a limit cycle which can be entrained by environmental cues, *e.g.*, sunlight. Disruptions to the circadian mechanism can lead to sleep and seasonal affective disorders [4].

Circadian rhythm represents only one example of oscillatory systems in biology. Another important oscillating system is the cell cycle. However, the key attributes of these systems, including period and phase, do not directly fit into the traditional framework of sensitivity analysis. Thus, the analysis of these systems requires an alternative approach that directly addresses the oscillatory behavior. One approach is based on the concept of the isochorn, the phase level set [5]. In biological systems, isochrons have been used extensively in the investigation of neural dynamics (see for example [6, 7, 8]). The concept has also been applied to illustrate phase resetting in circadian rhythm [9]. The usual approach employs a phase model or a coordinate

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transformation to phase variables [5, 7], which may pose a difficult problem for larger mechanistic models.

This work presents systems theoretic tools, based on the isochron, for analyzing the phase response of oscillatory systems to perturbations in system parameters. The tools will apply directly to the state differential equations without the need for a coordinate change. Two phase response analyses with respect to infinitesimal and finite parameter variations are presented. The former builds on the analysis developed for oscillatory chemical systems by Kramer *et al.* [10], with novel utilities to different phase response measures (such as the PRC). The latter method provides a phase analysis with respect to finite parameter perturbations (such as in modeling light input in the circadian rhythm [11]). The utilities of these analyses are demonstrated using a mechanistic *Drosophila* circadian rhythm model [12].

2 Isochrons

Before discussing the phase response analysis, it is important to define the meaning of "phase". The phase ϕ in a limit cycle refers to the (relative) position along the orbit, which is here measured by the elapsed time (modulo the period) to go from a reference point to the current position on the limit cycle (see Figure 1). Consequently, time and phase are interchangeable when the trajectory is on the limit cycle. In addition, the phase difference between two trajectories can be defined as the difference in the time it takes for each trajectory to achieve the same phase in the limit cycle as illustrated in Figure 1 (right). By this definition, a positive phase difference implies a phase lag and a negative value implies a phase lead.

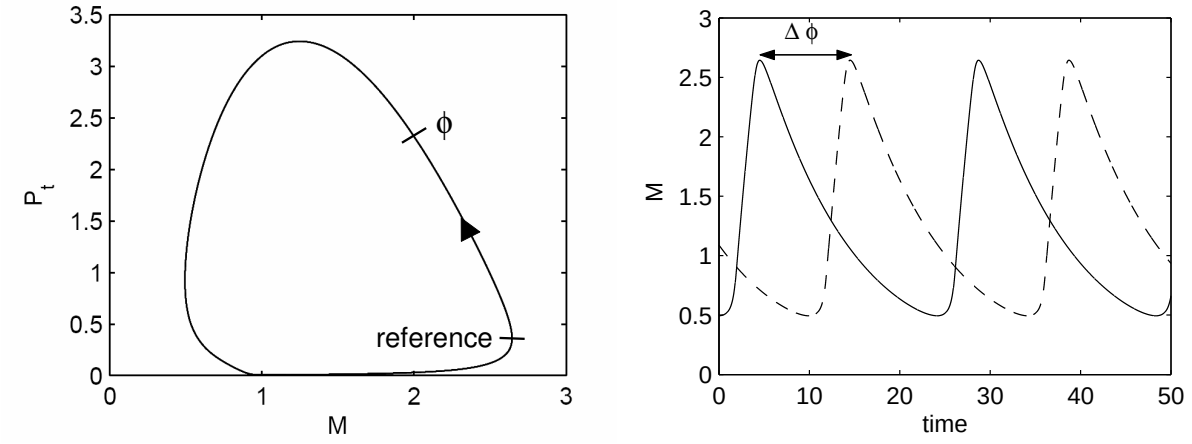

Fig. 1: (Left) An simple asymptotically stable limit cycle. The phase reference is arbitrary. (Right) Two trajectories in a limit cycle with a phase difference of $\Delta\phi$; the solid trajectory leads the dashed, or vice versa, the dashed trajectory lags the solid.

The enabling concept in the current work is the phase level sets, known as *isochrons*. An isochron of a limit cycle is a set of points in $\mathbf{R}^{n-1}$ from which state trajectories evolve to the same phase on the limit cycle (as $t \to \infty$). In a 2-state system, the isochrons $\eta(t)$ appear as lines

traversing the limit cycle as illustrated in Figure 2. Naturally, the isochron $\eta(t)$ overlaps with the isochron $\eta(t+k\tau)$ where k is an integer and τ is the natural period. Given two points in the basin of attraction of a limit cycle (not necessarily on the orbit), one can directly compute the phase difference based on the time difference of the isochrons to which these points belong. This phase definition is equivalent to measuring the time difference between two trajectories to reach the same isochron. Thus, the isochrons act as phase grids of a limit cycle. Direct computation of the isochrons is prohibitively expensive, especially in higher order systems. One method entails discretizing the basin of attraction, simulating the system from each discretization point, and collecting the states at every integer multiple of the period. Another method for computing isochrons involves backward integration of the system starting from the limit cycle, and again collection of points at a time interval of the period. Fortunately, the proposed analyses here do not require the full mapping of the isochrons.

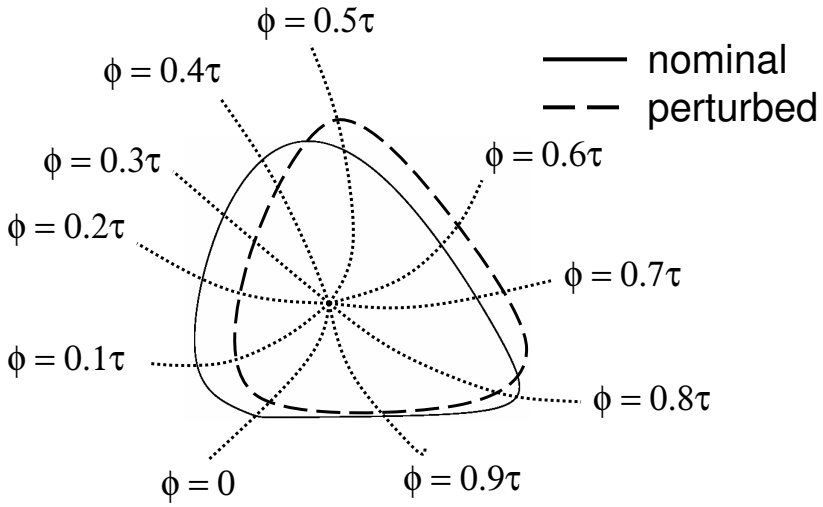

Fig. 2: A hypothetical 2-state limit cycle model and a corresponding perturbed limit cycle. The role of isochrons is to effectively map the state space into one variable phase axis. The phase of other limit cycles in the same state space can be measured using the nominal isochrons.

3 Phase Sensitivity Analysis

The system considered in this work is described by coupled ordinary differential equations:

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x}, t, \mathbf{p}) \tag{1}$$

where $\mathbf{x} \in \mathbf{R}^n$ denotes the states, $\mathbf{p} \in \mathbf{R}^m$ the parameters, t the time, and $\mathbf{f}$ is a vector of (nonlinear) functions of the states, time, and parameters. The states typically represent the mRNA and protein concentrations, and the parameters consist of the kinetic constants of different processes such as transcription, translation, and phosphorylation. The state trajectory $\mathbf{x}(t)$ is assumed to evolve to an asymptotically stable limit cycle (a closed trajectory in the state space), independent of the initial conditions.

The phase response with respect to infinitesimal variations in initial condition (IC) represents the simplest phase analysis, but is necessary for the development of more complicated parametric sensitivity. The effect of changes in IC is only transient since the perturbed trajectory will approach the nominal limit cycle. Thus, the phase difference corresponds to the isochron jump due to the perturbation. Since the computation of isochrons is computationally prohibitive, the phase shift is instead measured on the limit cycle, giving the following formulation: [10]

$$Q_j(0) = \frac{\partial \phi}{\partial x_j(0)} = -\lim_{t' \to \infty} \left(\frac{\partial x_i(t')}{\partial x_j(0)} \right) \Big/ \left(\frac{dx_i(t')}{dt} \right) \tag{2}$$

where Q_j is the IC phase sensitivity with respect to $x_j(0)$. The limit in (2) highlights the fact that the trajectory can only asymptotically approach the limit cycle. Numerically, the limit should not pose a problem for many systems as the phase sensitivities can be computed to sufficient accuracy after a few cycles around the orbit.

The phase analysis of parametric variations poses a higher degree of difficulty as perturbations in the parameters will generally give different limit cycles, and the comparison of phase between two different limit cycles is problematic. As noted in the previous section, the phase difference at time t between the perturbed and nominal trajectories can be defined as the time difference of each trajectory to reach the isochron $\eta(t)$. Application of the same concept on the parameter perturbations allows the formulation of parametric phase sensitivity [10]

$$\left(\frac{\partial \phi(t)}{\partial p_j} \right)_\eta = \sum_{i=1}^{n} Q_i(t) \frac{\partial x_i(t)}{\partial p_j} \tag{3}$$

The subscript η signifies that the parametric phase sensitivity is measured in reference to a given isochron, $\eta(t)$. Equation (3) suggests that the parametric phase sensitivity reflects the (cumulative) phase shifts between the perturbed and nominal trajectories from the same initial condition. Figure 3 illustrates the interpretation of (3). Note that the phase difference between the perturbed and nominal trajectories is here measured on the nominal limit cycle.

When a parameter perturbation causes a change in the period, the parametric phase sensitivity in (3) diverges as the phase difference accumulates for every cycle around the orbit [13]. The rate of accumulation around each cycle is equal to the period change, which provides a method to quantify the period sensitivities from (3)

$$\frac{\partial \tau}{\partial p_j} = \left(\frac{\partial \phi(t+\tau)}{\partial p_j} \right)_\eta - \left(\frac{\partial \phi(t)}{\partial p_j} \right)_\eta \tag{4}$$

where t is sufficiently large to exclude transient behavior. The removal of the period change effects from the phase sensitivities provides the local variations of phase

$$\left(\frac{\partial \phi(t)}{\partial p_j} \right)_\tau = \left(\frac{\partial \phi(t)}{\partial p_j} \right)_\eta - \frac{t}{\tau} \frac{\partial \tau}{\partial p_j} \tag{5}$$

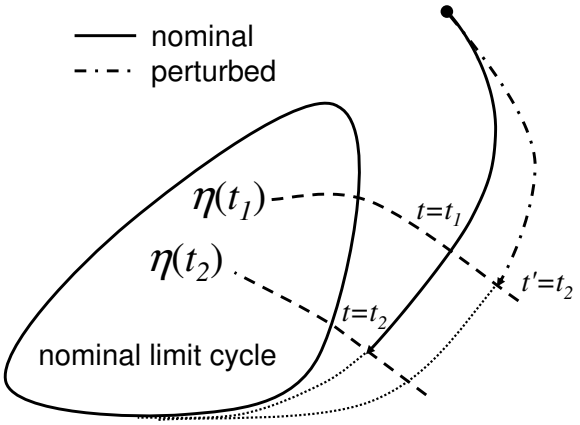

Fig. 3: Phase sensitivity with respect to the model parameters. The phase difference due to a parameter perturbation is $\Delta\phi = t_2 - t_1$. The dotted lines correspond to trajectories with nominal parameters, which imply that the phase difference is measured on the nominal limit cycle.

4 Phase Response Analysis

Sensitivity analysis captures the system changes to infinitesimal variations in the parameters (including initial conditions). However, the linear sensitivity coefficients may become inadequate for larger parameter perturbations due to the nonlinearity of the systems (a limit cycle model is inherently nonlinear). In practice, finite parameter perturbations are used to model different inputs to the limit cycle system, such as light entrainment in circadian rhythm [11] or gene knock-outs [14]. As mentioned above, parameter perturbations can change the limit cycle to which the states evolve. Nevertheless, the phase response to finite parameter perturbations can still be evaluated using the same isochron-based approach. Figure 2 illustrates a hypothetical limit cycle model in which one of the parameter is perturbed.

The following algorithm outlines the computation of the phase response to a finite parameter perturbation:

1. Generate the perturbed limit cycle (*i.e.*, the system with one or more of its parameters perturbed).
2. Discretize the perturbed limit cycle (usually equally spaced in time).
3. From each discretized point, simulate a nominal trajectory (the system with the original parameters) to approach the nominal limit cycle. The simulation length is selected to be an integer multiple of the nominal period. This length varies from system to system according to the strength of attraction to the limit cycle (for the circadian rhythm model used here [12], the trajectories approach the nominal limit cycle to within a defined numerical tolerance in 5 cycles).
4. Record the state vector at the final time and associate this information with the initial condition. Each of the perturbed-nominal state vectors belong to the same isochron.

5. Select one pair of perturbed-nominal states from item 4 as the reference pair. From the nominal reference, compute the phase (time distance) to the remaining nominal states identified in item 4 in sequence. (Note that the perturbed phases are equally spaced by design in item 2).

6. Compute the phase response, $\Delta\phi$, by subtracting the nominal phases in item 5 from the corresponding perturbed phase.

As in the above sensitivity analysis, the phase response can be normalized to the magnitude of the parameter perturbation, which constitute a finite difference approximation [15]. Also, the period change can be obtained from the phase response after one period around the limit cycle.

5 Phase Response Curve

In circadian rhtyhm, the efficacy of an entraining agent depends on the time at which it is administered. This efficacy is summarized in a phase response curve (PRC) which gives the phase shift induced by a pulse of entraining agent at different phases in a circadian clock. The PRC represents the dynamical aspect of the phase response. By definition, an entraining agent needs to modify the phase of the endogeneous oscillator. For this reason, modeling environmental cues typically involves perturbations on one or more parameters [11]. If the perturbation is sufficiently small and/or the system behaves considerably linear, the parametric phase sensitivity provides an avenue to compute the PRC. In this case, the PRC, $\rho(t)$, represents the accumulated phase shift over the duration of the entrainment effect:

$$\rho(t) = \sum_{j=1}^{r} \left[\left(\frac{\partial \phi(t + \Delta\theta)}{\partial p_j} \right)_{\eta} - \left(\frac{\partial \phi(t)}{\partial p_j} \right)_{\eta} \right] \Delta p_j \tag{6}$$

where $\Delta\theta$ denotes the effective duration of parameter changes caused by the resetting pulse, r is the total number of parameters affected by the entrainment, and Δp_j represents the magnitude of parameter change due to entrainment.

The algorithm for computing the phase response to finite perturbations gives an alternative, and more accurate, method to compute the PRC. Here, the perturbed limit cycle corresponds to the system with constant exposure to the entraining cue. The PRC can be computed using a similar formula

$$\rho(t) = \Delta\phi\left(\mathbf{p} + \mathbf{\Delta p}, t + \Delta\theta\right) - \Delta\phi\left(\mathbf{p} + \mathbf{\Delta p}, t\right) \tag{7}$$

where $\Delta\phi\left(\mathbf{p} + \mathbf{\Delta p}, t\right)$ is the phase response to finite parameter perturbations $\mathbf{\Delta p}$.

6 *Drosophila* Circadian Rhythm Case Study

The case study uses a model of the *Drosophila* (fruit fly) circadian rhythm gene network [12].

The circadian clock in a fruit fly consists of a gene regulation circuit where the key genes' transcriptions: period (*Per*) and timeless (*Tim*), are repressed by their own proteins (PER and TIM) [16], as illustrated in Figure 4. The regulation produces autonomous oscillations of mRNA and protein concentrations because of the effective delay between the transcription of mRNAs and the nuclear translocation of repressor proteins [2]. In *Drosophila*, the circadian clock exists mainly in lateral neurons in the central brain [16].

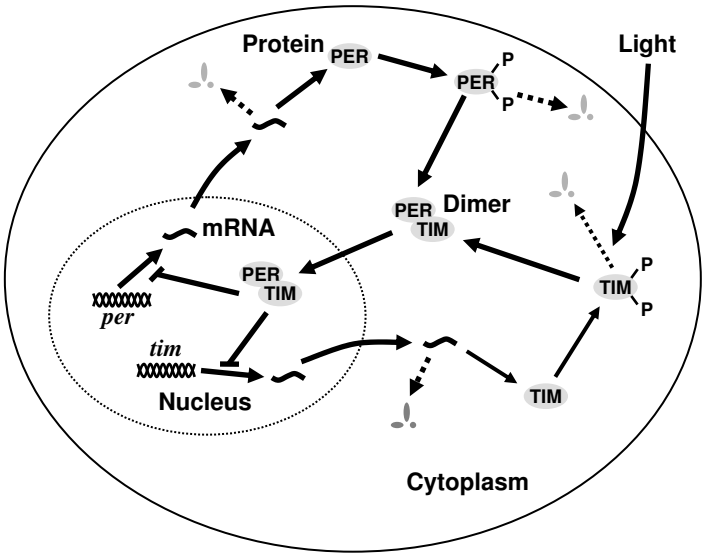

Fig. 4: An overview of *Drosophila* circadian rhythm gene regulation. The key genes are *Per* and *Tim* which produce the proteins PER and TIM. In the cell, the proteins can become prosphorylated and degraded or form the dimer PER-TIM which in turn inhibits the transcription of *per* and *tim* in the nucleus. Light increases the rate of degradation of TIM protein.

Light entrainment necessitates modeling the transcriptional regulation of both key proteins PER and TIM, since light selectively promotes the degradation of TIM [17, 18]. The example model has 10 states (two mRNAs, two proteins, two phosphorylated forms of each protein, and cytoplasmic and nucleic dimers) with 38 model parameters (not shown here for brevity) [12]. The phase shifts arise from an increase in the rate constant of TIM degradation, where a 10 minute light pulse is assumed to double the rate constant for a duration of 3 hours [11]. Figure 5 shows the autonomous oscillatory response of the model in absence of light.

The inclusion of light as an input in the model allows the construction of PRCs from the phase analyses. Figure 6 presents the phase sensitivities for four representative parameters, including the degradation kinetics of TIM. Similarly, Figure 7 shows the phase analysis based on a finite perturbation of the TIM degradation constant, *i.e.*, the perturbed limit cycle is under constant light input. Finally, Figure 8 compares the PRCs for a 10 minute light pulse ($\Delta\theta =$ 3 hours) computed from the results in Figures 6 and 7 [11]. As light induces a strong effect on the TIM degradation (100% perturbation in the rate constant), local sensitivity analysis may give inaccurate prediction of the phase shifts due to the nonlinearity of the system. This gives an explanation for the superior accuracy of phase analysis using finite perturbation over infinitesimal

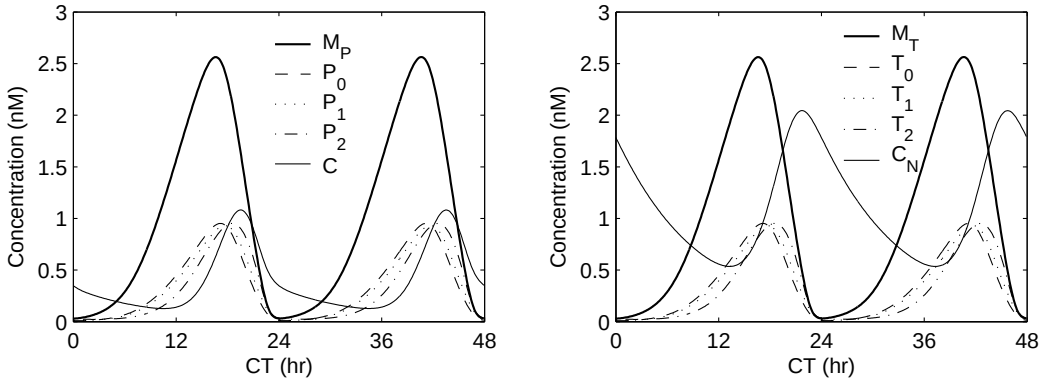

Fig. 5: Oscillatory behavior of a mechanistic model of *Drosophila* circadian rhythm. The model includes both the *per* and *tim* mRNAs (M_P and M_T respectively), the proteins and their phosphorylated forms (P_0, P_1, P_2 for PER and T_0, T_1, T_2 for TIM), and the PER-TIM dimer complexes (cytoplasmic C and nucleic C_N).

analysis in predicting the PRC.

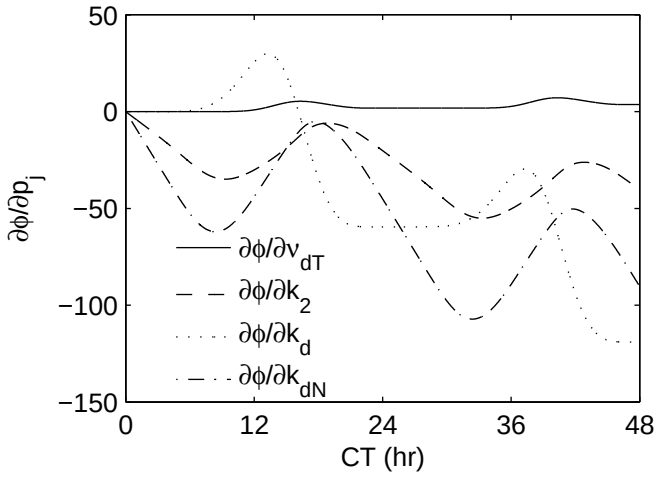

Fig. 6: Parametric phase sensitivities of the 10-state mechanistic *Drosophila* circadian rhythm model. The parameters k_2, K_{dP} (K_{dT}), k_d, and k_{dN} refer to the nuclear transport constant of the dimer PER-TIM, the Michaelis-Menten constant of PER (TIM) degradation, the degradation rate constant of proteins, and the degradation rate constant of nuclear dimer, respectively.

6 Discussion

Entrainment is the most important feature of a circadian rhythm. Unfortunately, this is also the least understood process in chronobiology [9, 20]. Past analyses on circadian rhythms focused on the period and amplitude of the oscillations to characterize the robustness of circadian behavior [21, 22]. However, period and amplitude analysis will have little use in comprehending

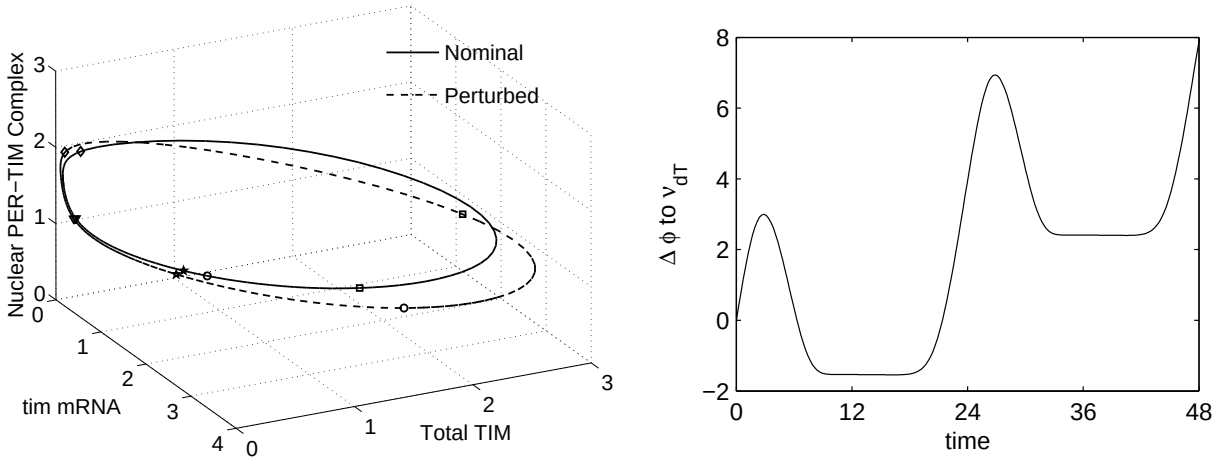

Fig. 7: (Left) Nominal and perturbed limit cycles of 10-state *Drosophila* model with representative pairs of points on the same isochrons. Each pair of perturbed-nominal states is marked by the same symbol. (Right) Phase response to a finite perturbation in the TIM protein degradation rate constant (ν_{dT}). The parameter is perturbed 100% of the nominal value to coincide with the assumed light entrainment strength.

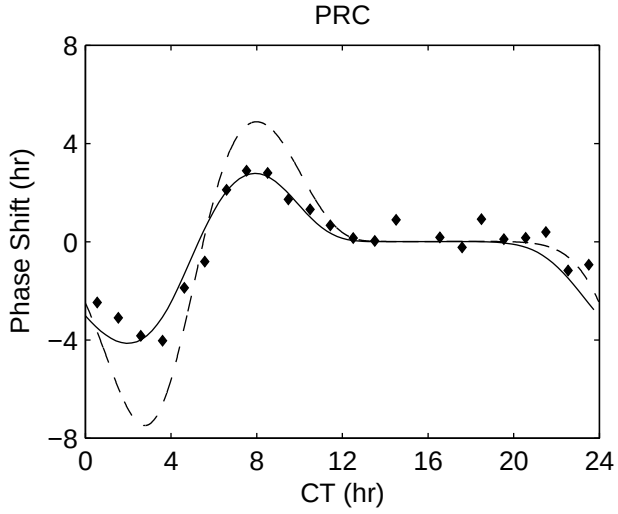

Fig. 8: Experimental and numerical PRCs of *Drosophila* circadian rhythm. The experimental data (filled diamond) were adapted from [19]. The predicted PRCs come from the phase response analysis of finite (solid) and infinitesimal (dashed) perturbations. In PRC, a positive value of phase shift indicates a phase advance and correspondingly, a negative value for a phase delay.

circadian entrainment because period sensitivity only measures cycle-to-cycle phase change, while amplitude sensitivity has no correlation with the phase. To elucidate the key mechanisms in circadian entrainment requires understanding the phase response to different perturbations in the systems. Figure 9 illustrates the lack of correlation between parametric sensitivities of the period and the phase, based on the 10-state circadian model. This result suggests that the circadian phase response is governed by a different set of processes (parameters) than the period modulation.

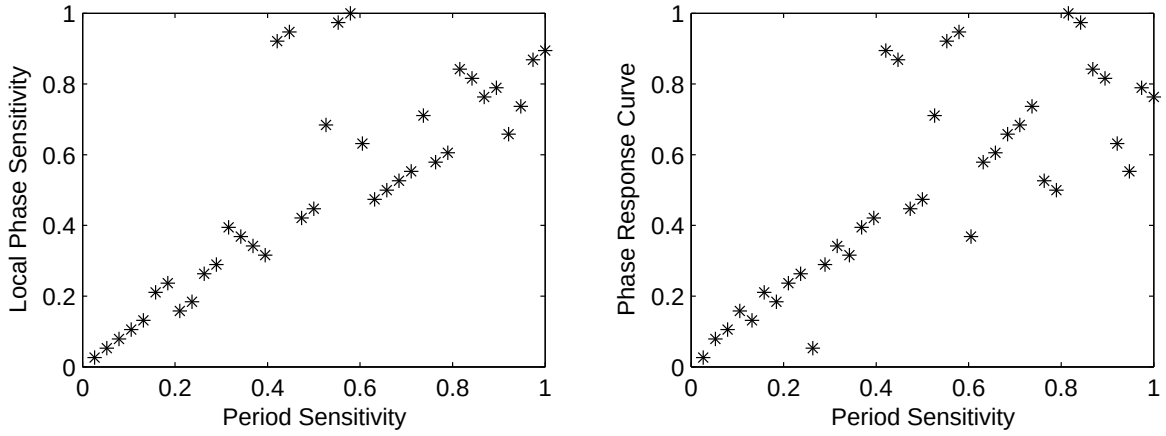

Fig. 9: Rank correlation between period and phase responses. The axes represent the normalized rank with the value 1 being the most sensitive. The local phase sensitivity is from (5) and the PRC is computed for a pulse of infinitesimal parameter perturbation (*i.e.*, the slope of parametric phase sensitivity). The phase rankings are based on the maximum magnitude of local phase sensitivity and PRC over one period.

To further classify the parameters based on their influence on the phase and period effects, Figure 10 presents the divisions in normalized parametric sensitivities for the phase and period. Quadrants I and III represent parameters which exert strong effect on either phase response or period modulation, respectively. On the other hand, quadrants II and IV contain parameters which have comparable effects on both the phase and period; comparably strong in II and comparably weak in IV. Parameters in quadrant I are consistently associated with the transcription of mRNAs (parameters ν_{sP}, ν_{sT}, K_{IP}, and K_{IT} in [12]). Quadrant III contains the parameters involved in the formation and nuclear transportation of PER-TIM dimers (parameters k_1,k_2, and k_3 in [12]). Finally, the parameters with comparably strong phase and period effects control the degradation of both mRNAs and proteins, and the protein translation (parameters ν_{mT}, ν_{mP}, ν_{dP}, ν_{dT}, k_{sP}, and k_{sT} in [12]).

The above classification of processes can be experimentally tested using genetic experiments that change the value of the associated parameters. For example, transcription rates can be varied by tuning the promoter strength using directed evolution [23], translation rates can be controlled using ribosomal binding sites of different strengths [24], and degradation kinetic of mRNA can be altered through modification of its secondary structure for stability [25]. The experiments can confirm or disprove the analysis based on the observed period versus phase changes. As an example, altering the transcription rates should give little change in the endoge-

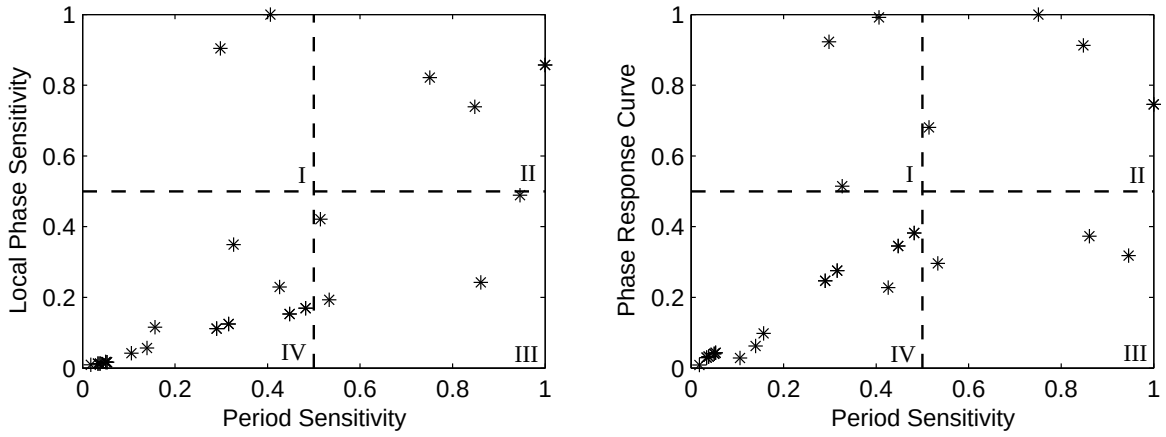

Fig. 10: Correlation between period and phase responses. The axes represent the normalized magnitude with respect to the largest among the parameters, such that the value 1 refers to the parameter with the largest magnitude. The parameters are grouped based on a 50% ratio to the largest sensitivity magnitude.

nous period, but large shifts in the phase response, such as the relative timing of the peaks and/or troughs of mRNAs and proteins. Another testable hypothesis in *Drosophila* circadian rhythm is that phase and period responses will have comparable roles in light entrainment, because light input increases the TIM degradation rate (parameter ν_{dT} in quadrant II). Evidence in the literature supported the contributions of both phase and period responses in the photic entrainment of circadian rhythm [26, 27], in agreement with this hypothesis.

6 Conclusion

The most important function of a circadian rhythm relies on the system phase response to synchronize its endogenous phase with the entraining cue, such as light. The phase analyses presented in this work offer a method for quantifying the dependence of phase response on the system parameters using isochron-based phase measures. Application of the analyses on a mechanistic model of *Drosophila* circadian rhythm [12] produced the classification of processes in the circadian gene regulation based on their phase and period response contributions. In particular, the mRNA transcriptions were found to preferentially regulate the phase response of the *Drosophila* circadian model. In addition, photic entrainment in this system by modulating the TIM degradation, was identified to have comparable control over the phase and period responses, in agreement with literature evidence. The resulting classifications can be tested using genetic experiments to alter the kinetic of processes in the circadian gene regulation.

Acknowledgments

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