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Phase Sensitivity Analysis of a Circadian Rhythm Gene Network

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Abstract— **Circadian rhythm synchronizes the daily activities of living organisms with the 24-hour earth rotation. The core of this clock is a transcriptional regulation of genes which produces an autonomous oscillation of 24-hour period. Despite the prevalence of oscillatory systems, sensitivity analysis of the key characteristics, such as period and phase, does not directly fit into the traditional framework for differential equations, which motivates the development of analyses aimed for these systems. This work focuses on the phase sensitivity analysis of circadian rhythm. Many of the concepts however can apply to oscillatory systems in general. Two examples of *Drosophila* circadian models illustrate the utilities of the developed analysis.**

I. INTRODUCTION

Circadian rhythm governs the day and night living cycle of many different species, from unimolecular Neurospora to highly multicellular mammals, as an adaptation to the 24-hour earth rotation. The structure of the circadian gene network is remarkably preserved across different species suggesting an evolutionary convergence [1]. The rhythm manifests itself in overt behavior such as the rest-activity cycle (sleep-wake), and controls many hormonal, physiological, and psychomotor performance functions [2]. The core of this rhythm consists of an autonomous genetic oscillator with multiple feedback loops forming a limit cycle which can be entrained by environmental cues, *e.g.*, sunlight. Disruptions to the circadian mechanism can lead to sleep disorders and possibly seasonal affective disorders [3].

Biological systems, including circadian rhythm, are known to exhibit robustness to external and internal disturbances, such as temperature fluctuations (external) [4] and inherent stochastic noise in gene regulation (internal) [5]. Here, robustness constitutes the ability of biological organisms to maintain certain phenotype under uncertainties in the environment. In circadian rhythm, the robustness of the period as a phenotype has been investigated using sensitivity analysis to elucidate the dependence of the states and period on the system parameters [6], [7]. Another key phenotype in a circadian rhythm is the (relative) phase of the circadian clock which describes the relative position in the limit cycle. Many circadian disorders arise because of the inability of certain organism to entrain its internal circadian clock to match the light-dark phase of the environment. Also, the efficacy of an environmental cue for entrainment depends on the phase at which the cue was administered. An example of such dependence appears in the phase response curve (PRC) which quantifies the phase advance/lag induced by a light pulse treatment [2]. Though arguably of higher importance, there has been little study on the phase sensitivity of circadian rhythms [8].

Oscillatory behavior are commonly encountered in biological systems such as the cell cycle, neuronal activity, and circadian rhythm. The key properties of these systems, including period and phase, do not directly fit into the traditional framework of sensitivity analysis. This work presents systems theoretic tools for investigating sensitivity of biological oscillatory systems, using the circadian rhythm system as an illustrative example. The tools are based on the phase sensitivity analysis for oscillatory chemical systems developed by Kramer *et al.* [9]. Novel utilities are developed to analyze different characteristics of the circadian clock including the PRC.

II. PHASE SENSITIVITY ANALYSIS

Sensitivity analysis elucidates the dependence of a system behavior on the parameters that affect the dynamics. First-order sensitivity coefficients provide the simplest measure of this dependence by quantifying the variations in the system outputs due to perturbations in the parameters:

$$S_{i,j} = \frac{\partial y_i}{\partial p_j} \tag{1}$$

where $S_{i,j}$ is the sensitivity coefficient of the i-th system output y_i with respect to the j-th parameter p_j. Although this definition implicitly assumes the continuity of the output with respect to the parameters, sensitivity analysis has been developed for systems in which this assumption does not hold, such as discrete stochastic systems [10]. The system outputs typically comprise of the states or some functions of the states. However, in a circadian rhythm, the quantities of interest including period and phase can not be easily represented as a function of the states. This necessitates developing a different type of sensitivity analysis for oscillatory systems [9].

The oscillatory system of interest is described by coupled ordinary differential equations:

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x}, t, \mathbf{p}) \tag{2}$$

where $\mathbf{x} \in \mathbf{R}^n$ denotes the states, $\mathbf{p} \in \mathbf{R}^m$ the parameters, t the time, and $\mathbf{f}$ consists of (nonlinear) functions of the states, time, and parameters. The trajectory $\mathbf{x}$ is assumed to evolve

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to a limit cycle independent of the initial conditions. There exist several methods to compute the sensitivity coefficients from (2) such as Direct, Green's function, and finite difference methods [11]. The Direct and Green's function methods obtain the sensitivities by solving the derivative of (2) with respect to each parameter

$$\frac{d}{dt}\frac{\partial \mathbf{x}}{\partial p_j}(t) = \mathbf{J}(t)\frac{\partial \mathbf{x}}{\partial p_j}(t) + \frac{\partial \mathbf{f}}{\partial p_j}(t) \tag{3}$$

where $\mathbf{J}(t)$ is the Jacobian matrix of $\mathbf{f}$ with respect to $\mathbf{x}$ (*i.e.*, $J_{i,j} = \partial f_i/\partial x_j$). The initial conditions are typically zero except when p_j is an initial condition of (2). The latter method solves a different differential equation for the Green's function matrix $\mathbf{\Gamma}(t,t')$

$$\frac{d}{dt}\mathbf{\Gamma}(t,t') = \mathbf{J}(t)\mathbf{\Gamma}(t,t'), \quad t \geq t' \tag{4}$$

with the initial condition $\mathbf{\Gamma}(t',t') = I$. The Green's function matrix provides the sensitivities according to

$$\frac{\partial \mathbf{x}}{\partial p_j}(t) = \mathbf{\Gamma}(t,0)\frac{\partial \mathbf{x}}{\partial p_j}(0) + \int_0^t \mathbf{\Gamma}(t,t')\frac{\partial \mathbf{f}}{\partial p_j}(t')dt' \tag{5}$$

Since t' is the integrating variable, the adjoint equation of (4) is a more practical system to solve:

$$\frac{d}{dt'}\mathbf{\Gamma}^\dagger(t',t) = -\mathbf{\Gamma}^\dagger(t',t)\mathbf{J}(t), \quad t' \leq t \tag{6}$$

where $\mathbf{\Gamma}^\dagger(t',t) = \mathbf{\Gamma}(t,t')$ and the initial condition is $\mathbf{\Gamma}^\dagger(t,t) = I$. Thus the adjoint Green function $\mathbf{\Gamma}^\dagger(t,t)$ must be solved backwards in time t'. The Green's function method becomes more efficient than the Direct method when $m > n$.

Before describing in detail phase sensitivity, it is important to define the meaning of "phase". Here, the phase ϕ in a system exhibiting a limit cycle refers to the (relative) position along the orbit which is measured by the elapsed time (modulo the period) to go from the reference point to the current position on the limit cycle. Consequently, time and phase are interchangeable when the trajectory is on the limit cycle. Based on this definition, 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 Fig. 1. Note that a positive phase difference implies a phase lag and a negative value implies a phase lead.

A. Sensitivity to Initial Conditions and Isochrons

The next two sections summarize the results of [9] on the sensitivity analysis of oscillatory chemical systems. The first type of sensitivity considered here is the phase sensitivity with respect to the initial conditions. Perturbations in the initial conditions can exert only transient effects on the system, *i.e.*, the perturbed trajectory will eventually approach the same limit cycle as illustrated in Fig. 2. In this case, the phase sensitivity is simply given by the phase difference between the nominal and perturbed trajectories normalized by the magnitude of the perturbation. A mathematical description of this definition follows

$$Q_j(0) = \frac{\partial \phi}{\partial x_j(0)} = -\lim_{t'\to\infty}\left(\frac{\partial x_i(t')}{\partial x_j(0)}\right)\Big/\left(\frac{dx_i(t')}{dt}\right) \tag{7}$$

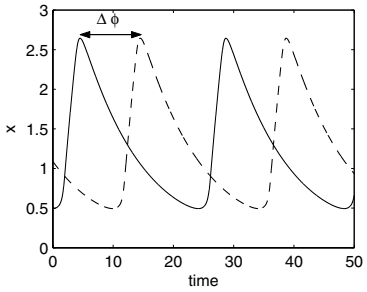

Fig. 1. Two trajectories in a limit cycle with a phase difference of $\Delta\phi$; the solid trajectory leads the dashed, or equivalently, the dashed trajectory lags the solid.

where Q_j are the phase sensitivity coefficients. The limit in (7) is necessary because 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 (typically 3 to 4 cycles). A more efficient method to compute the phase sensitivity follows from the Green's function method

$$Q_j(t) = -\lim_{t'\to\infty}\mathbf{\Gamma}^\dagger_{i,j}(t,t')\Big/\left(\frac{dx_i(t')}{dt}\right) \tag{8}$$

which requires the computation of only one row of the adjoint Green's function matrix $\mathbf{\Gamma}^\dagger_{i,j}$. Note that (8) gives not only the phase sensitivity coefficients with respect to perturbations of the states at initial time $t = 0$ but also to those at any given time t.

Given the phase sensitivity to initial conditions, it is possible to compute the combination of perturbations in the states such that the cumulative phase difference is zero

$$\sum_{j=1}^{n} Q_j(t)dx_j(t) = 0. \tag{9}$$

An isochron describes the collection of points satisfying (9) from which trajectories evolve to the same phase in the orbit as the nominal trajectory starting from $\mathbf{x}(t)$. In a 2-state system, these isochrons $\eta(t)$ appear as lines traversing the limit cycle as illustrated in Fig. 3. As expected, the isochron $\eta(t)$ overlaps with the isochrons $\eta(t + k\tau)$ where k is an integer and τ is the period. Though the isochrons here are defined based on the phase sensitivity to initial conditions, the converse is also fitting; the phase sensitivity to initial conditions is the time difference between the perturbed and nominal trajectories to reach a given isochron normalized by the perturbation size.

B. Sensitivity to Parameters

The phase sensitivity with respect to the model parameters $\mathbf{p}$ poses a higher difficulty as perturbations in the parameters will give different limit cycles from the nominal parameters and the comparison of phase between two different limit cycles is problematic. However, as noted in the previous section, the phase difference at time t between the perturbed and nominal trajectories is equal to the time difference

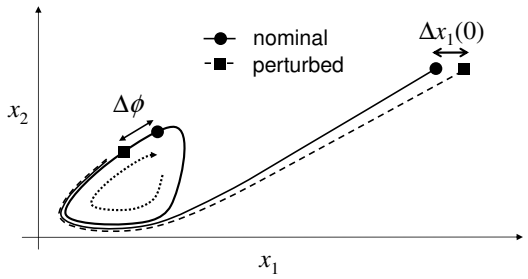

Fig. 2. Phase sensitivity with respect to initial conditions in a 2-state system.

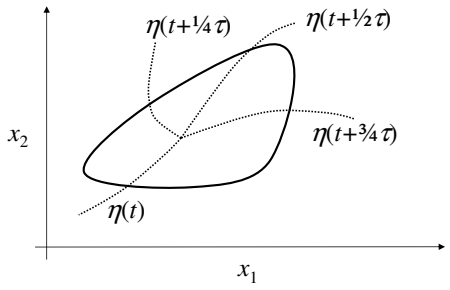

Fig. 3. A hypothetical limit cycle with the isochrons spaced equally in time.

of each trajectory to reach the isochron $\eta(t)$. Application of the same concept for the model parameters allows the formulation for the parametric phase sensitivity

$$\left(\frac{\partial\phi(t)}{\partial p_j}\right)_\eta = \sum_{i=1}^{n} Q_i(t)\frac{\partial x_i(t)}{\partial p_j} \tag{10}$$

The subscript η signifies that the phase sensitivity is measured in reference to a given isochron $\eta(t)$. Equation (10) indicates that the parametric phase sensitivity reflects the accumulated phase shifts arising from the change in the trajectory due to the parameter perturbations. Fig. 4 illustrates the derivation of the phase sensitivity with respect to the model parameters. Note that the phase difference between the perturbed and nominal trajectories is measured on the same (nominal) limit cycle.

Remark: When a parameter perturbation causes a change in the period, the parametric phase sensitivity (10) diverges as the phase difference accumulates for every cycle around the orbit. The rate of accumulation around each cycle is equal to the period change, which provides a method to quantify the period sensitivities from (10)

$$\frac{\partial\tau}{\partial p_j} = \left(\frac{\partial\phi(t+\tau)}{\partial p_j}\right)_\eta - \left(\frac{\partial\phi(t)}{\partial p_j}\right)_\eta \tag{11}$$

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

$$\left(\frac{\partial\phi(t)}{\partial p_j}\right)_\tau = \left(\frac{\partial\phi(t)}{\partial p_j}\right)_\eta - \frac{t}{\tau}\frac{\partial\tau}{\partial p_j} \tag{12}$$

The parametric phase sensitivity reflects only one part of the raw sensitivity in (3). The remainder corresponds to

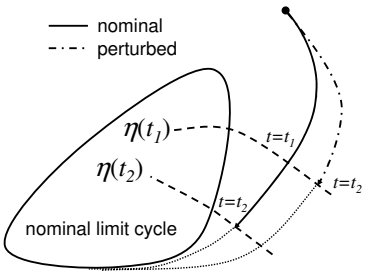

Fig. 4. Phase sensitivity with respect to the model parameters. The phase difference due to a parameter perturbation is $\Delta\phi = t_2 - t_1$. The dotted lines correspond to trajectories with nominal parameters, which imply that the phase difference is measured on the nominal limit cycle.

variations in the trajectory that lie on the isochron $\eta(t)$ as these variations do not produce a phase shift (referred to as path-independent). Fig. 5 illustrates an alternate derivation to [9] for the decomposition of raw sensitivity into the path-dependent and path-independent parts:

$$\frac{\partial x_i}{\partial p_j} = \left(\frac{\partial x_i}{\partial p_j}\right)_\eta - \left(\frac{\partial\phi}{\partial p_j}\right)_\eta \frac{dx_i}{dt} \tag{13}$$

A similar decomposition also exists [12] that separates the raw sensitivities into the shape and period changes

$$\frac{\partial x_i}{\partial p_j} = \left(\frac{\partial \mathbf{x}}{\partial p_j}\right)_\tau - \frac{t}{\tau}\frac{\partial\tau}{\partial p_j}\frac{dx_i}{dt} \tag{14}$$

C. Phase Response Curve

The efficacy of entrainment of circadian rhythm by environmental cues strongly depends on the time at which the resetting cue is administered. This efficacy is summarized in a phase response curve (PRC) which gives the phase shift induced by a particular cue at different phases in a circadian clock. The PRC represents the dynamical aspect of the phase sensitivities. Modeling of environmental cues typically involves perturbations on the parameter values for a finite duration of time [16], which falls within the framework of the parametric phase sensitivity above. In this case, the PRC $\rho(t)$ represents the accumulated phase shift:

$$\rho(t) = -\sum_{j=1}^{r}\left[\left(\frac{\partial\phi(t+\Delta\theta)}{\partial p_j}\right)_\eta - \left(\frac{\partial\phi(t)}{\partial p_j}\right)_\eta\right]\Delta p_j \tag{15}$$

where $\Delta\theta$ denotes the duration of parameter changes induced by the resetting cue, r is the total number of parameters affected by the entrainment, and Δp_j represents the magnitude of parameter change due to entrainment. In the PRC, a positive value denotes phase advancing, and thus the negative sign in (15) becomes necessary.

D. Alternate Definition of Phase

Aside from the definition used in the previous sections, the term phase can also describe the time separation between two isochrons in the state trajectories (commonly peaks and/or troughs) as illustrated in Fig. 6. Sensitivity analysis of this alternate phase $\hat{\phi}$ can still fit in the framework of the preceding phase sensitivity. However, the most intuitive

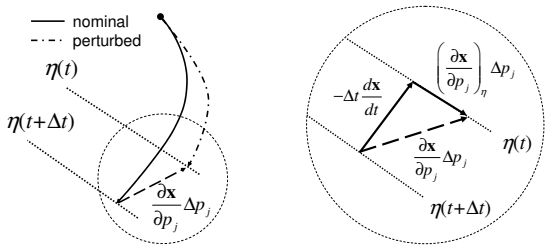

Fig. 5. A geometrical decomposition of raw sensitivity into path-dependent and -independent parts. The path-dependent portion $-\Delta t \frac{d\mathbf{x}}{dt}$ is equivalent to $-\Delta p_j \left(\frac{\partial \phi}{\partial p_j}\right)_\eta \frac{d\mathbf{x}}{dt}$.

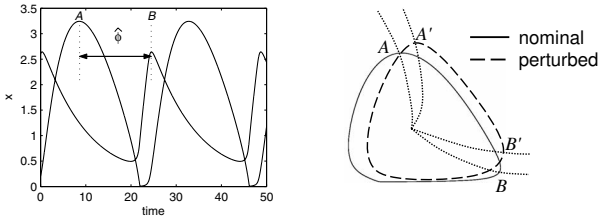

Fig. 6. Sensitivity analysis of the alternate phase $\hat{\phi}$ shown here as the peak-to-peak time separation. The parameter perturbations can produce changes in the shape of the limit cycle which shifts the peak-to-peak isochrons of $\hat{\phi}$ from A to A' and from B to B'.

method to derive the alternate phase sensitivity from (10) in the spirit of (11) and (15), proves to be incorrect. The complexity arises because the parameter perturbations induce shape changes in the limit cycle, and thus the reference isochrons of $\hat{\phi}$ in the perturbed limit cycle may shift from those in the nominal system (see Fig. 6). In this case, the parametric sensitivity of $\hat{\phi}$ reflects how long it takes the perturbed trajectory to travel from the isochrons A' to B' in comparison to the nominal trajectory to travel from A to B. The alternate phase sensitivity needs to correct for the aforementioned effect of shape changes

$$\frac{\partial \hat{\phi}}{\partial p_j} = \left(\frac{\partial \phi}{\partial p_j}\right)_\eta \Bigg|_{A'}^{B'} - \left[\sum_{i=1}^{n} Q_i \left(\frac{\partial x_i}{\partial p_j}\right)_\tau\right]\Bigg|_A^B \tag{16}$$

where

$$g(t)|_A^B = g(t_B) - g(t_A) \tag{17}$$

and $g(t)$ is some function of time. The last term in (16) takes into account the isochorn shifts due to the shape change in the limit cycle. The relationships between the isochrons follow

$$\begin{aligned} \eta(t_{A'}) &= \eta\left(t_A - \sum_{i=1}^{n} Q_i \left(\frac{\partial x_i(t_A)}{\partial p_j}\right)_\tau\right) \\ \eta(t_{B'}) &= \eta\left(t_B - \sum_{i=1}^{n} Q_i \left(\frac{\partial x_i(t_B)}{\partial p_j}\right)_\tau\right) \end{aligned} \tag{18}$$

III. EXAMPLES

The examples come from models of *Drosophila* (fruit fly) circadian rhythm gene networks; a simple 2-state model [13] and a 10-state mechanistic model with light entrainment input [14]. Circadian clock in a fruit fly consists of a gene transcription-translation regulation by feedback inhibition of key genes' transcription: period (*per*) and timeless (*tim*), by their own proteins (PER and TIM) as illustrated in Fig. 7 [4]. The regulation produces autonomous oscillations of mRNA and protein concentrations because of the time delay between the transcription of the mRNAs and the nuclear translocation of the repressor proteins [1]. In *Drosophila*, the circadian clock exists mainly in lateral neurons in the central brain [4].

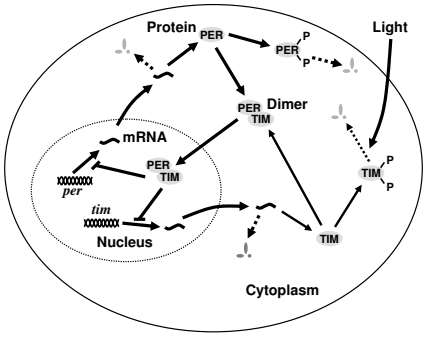

Fig. 7. An overview of *Drosophila* circadian rhythm. The key genes are *per* and *tim* which produce the proteins PER and TIM. In the cell, the proteins can become prosphorylated and then degraded, or form the dimer PER-TIM which in turn inhibits the transcription of *per* and *tim* in the nucleus. Light increases the rate of degradation of TIM protein.

A. 2-state Circadian Rhythm

One of the simplest models of the autonomous circadian rhythm [13] tracks only the mRNA and protein concentrations

$$\begin{aligned} \frac{dM}{dt} &= \frac{\nu_m}{1 + (P_t(1-q)/2P_{\text{crit}})^2} - k_m M \\ \frac{dP_t}{dt} &= \nu_p M - \frac{k_{p1} P_t q + k_{p2} P_t}{J_p + P_t} - k_{p3} P_t \\ q &= \frac{2}{1 + \sqrt{1 + 8K_{eq} P_t}} \end{aligned} \tag{19}$$

where M and P_t denote the mRNA (*per* or *tim*) and the protein (PER or TIM) concentrations respectively, and $[\nu_m,\ k_m,\ \nu_p,\ k_{p1},\ k_{p2},\ k_{p3},\ K_{eq},\ P_{\text{crit}},\ J_p]$ are the model parameters. For parameter values given in [13], Fig. 8 shows that the system has an asymptotically stable limit cycle with a period of approximately 25 hours.

The phase sensitivities (7) and (10) on the limit cycle are presented in Fig. 9. The period sensitivities can be directly calculated from the parametric phase sensitivity curves at $t = \tau$. Comparisons to the SVD method [12] in Table I confirms the accuracy of the period sensitivities. In fact, the phase sensitivities present the most accurate estimates of the period sensitivities among the existing techniques [12], [15] because the only source of inaccuracy comes from the simulation error (other methods involve additional numerical approximations). Using the alternate phase definition as the

TABLE I

PERIOD SENSITIVITIES OF 2-STATE CIRCADIAN MODEL

$\mathbf{p}$	$\partial\tau/\partial p_j$	$\partial\tau/\partial p_j$ of [12]
ν_m	2.5297	2.5064
k_m	-187.87	-188.18
ν_p	5.0452	6.7089
k_{p1}	-0.0399	-0.0921
k_{p2}	-31.775	-32.752
k_{p3}	-90.793	-93.415
K_{eq}	0.0028	0.0041
P_{crit}	48.088	47.363
J_p	-108.22	-105.36

TABLE II

ALTERNATE PHASE SENSITIVITIES

$\mathbf{p}$	$\partial\hat{\phi}/\partial p_j$	$\partial\hat{\phi}/\partial p_j$ using FD
ν_m	0.8543	0.4923
k_m	-63.457	-56.512
ν_p	1.7014	0.9846
k_{p1}	-0.0135	0.0223
k_{p2}	-10.724	-7.6604
k_{p3}	-30.635	-35.982
K_{eq}	0.0010	-0.0001
P_{crit}	16.241	3.6333
J_p	-36.517	-17.517

(smaller) time separation between the peaks of M and P_t ($\hat{\phi}_{\text{nominal}} \approx 8.3$ hours), Table II lists the alternate period sensitivities (16) which are in general agreement with the crude estimates from a finite difference approach (FD) [11].

B. 10-state Circadian Rhythm

The inclusion of light entrainment necessitates modeling the transcriptional regulation of both key proteins PER and TIM. The model consists of two negative feedback loops as shown in Fig. 7 and involves 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) [14]. The light induced phase shifts arise from an increase of the rate constant for TIM degradation, where a 10 minute pulse of light is assumed to double the rate constant as long as 3 hours [16]. Fig. 10 shows the autonomous oscillatory response of the model in absence of light.

The inclusion of light entrainment in the model allows the construction of the PRC from the phase sensitivities. Fig. 11 compares the PRC for a 10 minute light pulse ($\Delta\theta$ = 3 hours) computed from the parametric phase sensitivities and from direct perturbations on the degradation rate of TIM [16]. The discrepancies between the two PRCs arise because sensitivity in general represents only local perturbation on the parameter. Thus, the size of parameter perturbation due to the light pulse (100% perturbation) violates the locality assumption and introduces the observed discrepancies.

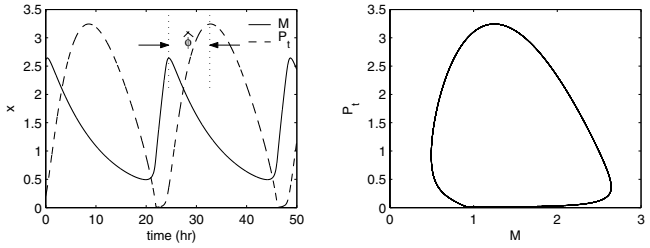

Fig. 8. *Drosophila* circadian rhythm model with an oscillatory response (left) and its corresponding limit cycle (right) [13].

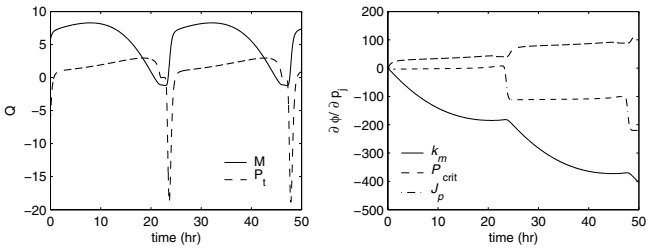

Fig. 9. Phase sensitivities of a 2-state circadian rhythm model. For brevity, only three of the parametric phase sensitivities were plotted.

IV. DISCUSSION

The system theoretic analysis developed in the previous sections allow computation of different key characteristics of circadian rhythm from only the phase sensitivity curves (10), including the period sensitivity and PRC. As the evaluation of the sensitivities involves (numerical) solutions of differential equations, the tools present the most accurate method to obtain both period and phase sensitivities. A recent work in the analysis of circadian rhythm [8] presented phase sensitivities as impulse response curves (IRCs), which quantify the phase shifts from an impulse parameter perturbation at different times. The IRCs and the present phase sensitivities curves are in fact equivalent; the slopes of the phase sensitivities (10) with respect to time give the IRCs.

The reason for defining the alternate phase in (16) is rooted in model identification. Parameter values for biological models are very difficult to obtain and thus, the magnitudes of $\mathbf{x}$ typically carry little predictive value. Therefore, data fitting needs to rely on relative measures such as the peak to peak

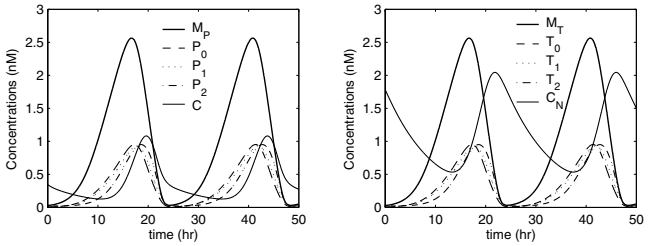

Fig. 10. Oscillatory behavior of a mechanistic model of *Drosophila* circadian rhythm [14]. The model includes both the *per* and *tim* mRNAs (M_P and M_T respectively), the proteins and their phosphorylated forms (P_0, P_1, P_2 for PER and T_0, T_1, T_2 for TIM), and the PER-TIM dimer (cytoplasmic C and nucleic C_N).

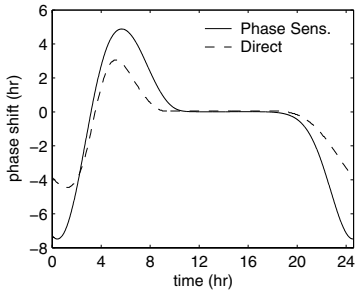

Fig. 11. Phase response curve of *Drosophila* circadian rhythm model computed from the parametric phase sensitivities and direct perturbations [17]. Here, positive value of phase shift indicates phase advance.

TABLE III
NORMALIZED PERIOD AND LOCAL PHASE SENSITIVITIES OF 2-STATE CIRCADIAN MODEL

$\mathbf{p}$	Norm. $\partial\tau/\partial p_j$	Max. Abs. of (12)
ν_m	2.5278	1.8147
k_m	-18.786	6.4156
ν_p	2.5277	15.961
k_{p1}	-0.4027	13.234
k_{p2}	-0.9533	0.3317
k_{p3}	-9.0794	1.5145
K_{eq}	0.5682	6.0497
P_{crit}	4.8070	2.1978
J_p	-36.517	5.5101

time separation in the case of circadian rhythm. For example, the peaks of protein level PER and TIM typically lag those of the mRNAs by 6 hours [4]. Here, the alternate phase sensitivities can direct the tuning of model parameters to match experimental observations.

The robustness properties of the circadian rhythm have been previously analyzed using the period and amplitude sensitivities [6]. However, light entrainment of a circadian clock is a phase resetting problem and does not correlate with the period and amplitude as the effect of light is only temporary. Since the period sensitivities capture only the cycle to cycle phase difference, the phase sensitivities may provide additional information to the period analysis. For example, comparisons of the normalized period sensitivities with the maximum local phase variations (12) for the 2-state circadian model show differences in the relative ranking of the parameters based on the sensitivity magnitudes (see Table III). In particular the system shows high phase sensitivity to k_{p1} with low period sensitivity, suggesting high efficacy of entrainment with little period change is possible by targeting this parameter. Such analysis may have physiological manifestations to help explain the robustness-fragility trade off in the evolution process [18] when applied to a complete model of biological (oscillatory) systems.

V. CONCLUSIONS

The developed tools provide an efficient and accurate analysis of key characteristics of circadian rhythm and other biological oscillatory systems. The enabling concept for the analysis is the isochorns, collection of points that evolve to the same position in the limit cycle, which allows quantification of phase shifts between different limit cycles. The analysis can be used to guide model identification as well as to elucidate the design principles guiding the evolution process.

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