The Third International Symposium on Optimization and Systems Biology (OSB'09)
Zhangjiajie, China, September 20–22, 2009

Stochastic Synchronization of Circadian Rhythms

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Abstract The models of circadian genetic oscillators involving interwined feedback processes in molecular level genetic network in *Drosophila melanogaster* and *Neurospora crassa* are studied and examine mechanisms whereby synchronization can arise in an assembly of cells. The individual subcellular circadian oscillatory processes are stochastic in nature due to small numbers of molecules are involved in the processes and large fluctuations subjected to them. We investigate and present the simulations of the stochastic dynamics of ensembles of clock–regulating proteins in different nuclei that communicate via ancillary small molecules, environmental parameters, cellular noise (additive), or through diffusive processes. The results show that the emergence of collective oscillations is a macroscopic observable which has its origins in the microscopic coupling between distinct cellular oscillators.

1 Introduction

Biological internal autonomous time keeping mechanisms allow all living organisms to adapt to external diurnal periodicity [3]. Circadian rhythms are based on specific genetic processes, and are known to originate in negative feedback circuits [4, 3, 6, 5] that generate a periodic expression of specific genes [7, 3] within a cell. Individual cells in the organism can thus be viewed as an autonomous oscillators that contain a genetic circuit that can couple among themselves and with various environmental parameters [11, 10]. The genetic oscillators based on some models involving various molecular mechanisms have been studied in organisms such as *D. melanogaster*, or *N. crassa*[1, 9, 10], but the larger question of how groups of cells exhibit rhythms synchronously is one that remains incompletely understood. Since synchrony is in fact essential for information processing in such systems [12], the phenomenon of synchronization can be seen, in general, as the consequence of cell–to–cell communication via specific coupling mechanisms [13]. The cellular synchrony can be achieved via various signaling molecules [13] or a number of internal or external stimuli [3, 6] and is a robust phenomenon.

The molecular processes that have been developed to model circadian oscillators [9] are studied in this work and examine different mechanisms for an ensemble of such oscillators to synchronize. For instance, in the fruitfly *D. melanogaster* the clock mechanism depends on the proteins CLOCK and CYC which induce rhythmic transcription of PER

and TIM, the latter two being negative feedback regulators [14, 15]. Experiments using *per* mutants have shown that changes in the structure or abundance of this gene product can lengthen, shorten or even completely remove the periodicity of circadian rhythms [16, 17, 18]. While PER and TIM are too large to diffuse out of a given cell, there is some evidence that they play the role of "couplers" when positioned near the cell membrane: they can then synchronize (or otherwise organize) clock components [19, 20]. The humoral peptide hormone (PDF) which is a *pdf* gene product secreted from individual cells of *D. melanogaster* is also believed to act as a synchronizing factor, inducing phase shifts in the same manner as a periodic light pulse [21]. *N. crassa*, on the other hand, has a syncitial morphology [3, 10]: several nuclei are present in a single cell, thus "sharing" a common cytoplasm. As a consequence, the clock gene mRNA and clock proteins are common to a set of clock oscillators and can transport directly from one oscillator to the other by diffusion or convection. The coupling between different oscillators is effected via these molecular mechanisms [10, 22].

The cellular and subcellular processes are dynamically diverse since there are a number of distinctly different oscillator types based on a variety of coupling topologies and architectures. At the same time it is also recognised that the temporal organisation of the different dynamical processes is crucial to the functioning of cells, and thus the manner in which these different oscillators interact with each other as well as with nonoscillatory processes is central to understanding temporal organization at the subcellular level. It is necessary to understand the manner in which synchronization takes place, and indeed the synchronizability of this diverse set of processes. Thus studies of the general mechanisms that may underlie synchrony in such systems is of considerable interest [23].

We focus on a stochastic model for circadian rhythms that has been extensively studied over the past few years [11] and examine how synchrony emerges in an ensemble of such stochastic oscillators. In the present work we have considered the oscillators to be similar in type, although there is variation in the fundamental parameters of the model; this is described in the next section of this paper where we also discuss the simulation methods we have used. The main results of this study are presented in Section III: we examine several coupling strategies and topologies, and using quantitative criteria, discuss the manner in which the phase synchronization of distinct oscillators can be judged. A summary and discussion follows in Section IV.

2 Models of circadian oscillator and simulation techniques

There are different theoretical models to explain molecular mechanisms in circadian oscillatioors [1], out of which one that is specific to *D. melanogaster* and *N. crassa* is a single feedback loop model developed by Goldbeter and coworkers [11, 24]. The model is based on negative feedback autoregulation of gene expression whose molecular mechanisms associated with the feedback loop in both the species are quite similar even even though the specific proteins involved are quite different as in Fig. 1 [8, 9].

The essential steps leading to oscillation of a given protein are as follows. Consider the *per* gene in *D. melanogaster*. Following its transcription, the corresponding *mRNA* (M_P) is transported into the cytosol where it is translated into protein P_0 and degraded. Reverse phosporylation can also take place from P_0 to P_1 and then P_2: this can be degraded or transported into the nucleus as the nuclear protein, P_N when it then represses *per*.

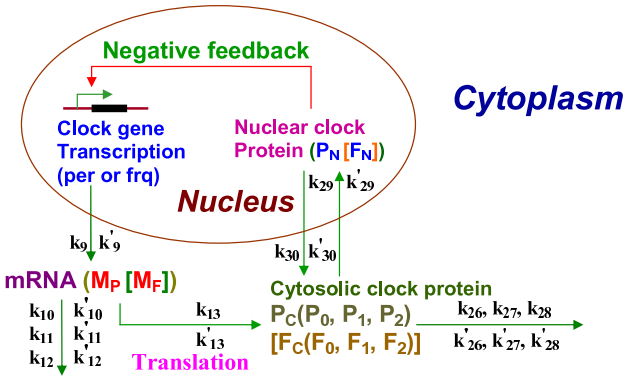

Figure 1: Circadian model developed by Goldbeter and co-workers [9, 10, 24].

In *N. crassa*, the operative protein is *frq*, and the same mechanism can be invoked to understand its oscillations. In this case, the transcript clock gene is replaced by $mRNA(M_F)$. The translated cytosol proteins and nuclear protein are now become F_0, F_1, F_2 and F_N respectively. The transcription rates, translation rates and degradation rates of $mRNA(M_P$ *and* $M_F)$ and cytosol protein (P_C and F_C) of the two organisms (see shown in Fig. 1) are different.

Dynamical processes at the subcellular level typically involve a small number of participating entities, and can be seen essentially as noise-driven stochastic processes [11, 25, 26]. We use stochastic simulation techniques [27, 28, 29] in the present study, considering the cell as containing N distinct molecular species $(X_1, X_2, \cdots, X_N)$ that react via M reaction channels R_μ with reaction constants $k_\mu, \mu = 1, 2, \ldots M$ such as

$$X_i + X_j + X_k + \cdots \xrightarrow{k_\mu} X_\alpha + X_\beta + X_\gamma + \cdots \tag{1}$$

where the X's are molecule populations. A configuration $\mathscr{C}$ is the population vector $(X_1, X_2, \cdots, X_N)$, and the configurational probability $P(\mathscr{C}, t)$ obeys the master equation [27],

$$\frac{dP(\mathscr{C}, t)}{dt} = -\sum_{\mathscr{C}'} P(\mathscr{C}, t) W_{\mathscr{C} \to \mathscr{C}'} + \sum_{\mathscr{C}} P(\mathscr{C}', t) W_{\mathscr{C}' \to \mathscr{C}} \tag{2}$$

where the W's are the appropriate transition probabilities. The above master equation is simulated via the Gillespie algorithm [27]; and also also considered some of the elementary processes to include time–delay [30].

3 Synchronization of stochastic oscillators

The variables in stochastic systems are in general subject to both intrinsic and extrinsic fluctuations. The synchronization of such two stochastic systems cannot be detected in a simple manner and the situation can be more complex if one deals with an ensemble of such systems [31]. Extensive studies of coupled deterministic dynamical systems

has revealed that there can be many forms of synchronization, ranging from complete synchrony, when all variables of the coupled systems are identical to generalized synchronization, when the variables of the two systems are unique functions of each other.

The phase synchronization becomes a relevent form in dealing with stochastic systems when the relevant variables oscillate together without having identical amplitudes [35]. We therefore discuss, the detection of phase synchronization between two signals [34], and general coupling schemes whereby such phase synchronization occurs.

The time evolution of two independent and identical stochastic systems starting with different initial configurations will be uncorrelated. Upon coupling, the subsystems can synchronize although one should not expect complete synchronization; due to the inherent stochasticity the subsystems show phase synchrony [33, 34, 35]: the variables do not coincide but vary in unison even when fluctuations are large [33, 35].

It has been pointed out [36] that it is possible to define an instantaneous phase for an *arbitrary* signal $\eta(t)$ via the Hilbert transform [34]

$$\tilde{\eta}(t) = \frac{1}{\pi} P.V. \int_{-\infty}^{+\infty} \frac{\eta(t)}{t-\tau} d\tau \tag{3}$$

where *P.V.* denotes the Cauchy principal value. Then, through the relation

$$A(t)e^{i\phi(t)} = \eta(t) + i\tilde{\eta}(t), \tag{4}$$

one can associate an instantaneous phase $\phi(t)$ and an instantaneous "amplitude" $A(t)$ with a given signal. Given two signals, one can therefore obtain the instantaneous phases ϕ_1 and ϕ_2; phase synchronization is then the condition that $\Delta\phi = m\phi_1 - n\phi_2$ is constant. Of most interest are the cases $\Delta\phi = 0$ or π, namely the cases of in–phase or anti–phase, but other temporal arrangements may also occur.

We now consider the coupling schemes that lead to the synchronization of two systems. In what follows we will denote the configurations of the the two essentially identical systems by $\mathscr{C} \equiv (X_1, X_2, \cdots, X_N)$ and $\mathscr{C}' \equiv (X_1', X_2', \cdots, X_N')$ respectively. The following forms of coupling have been discussed [35].

1. **Direct coupling**: The two subsystems share one species in common, say X_i and X_i' are identical. Then the dynamics of the remaining variables (X_j and X_j') become highly correlated. In the deterministic limit, this reduces to an analogue of the master–slave coupling [37].
2. **Exchange or Diffusive coupling**: In this scenario, the species X_i and X_i' can interconvert via additional reaction channels, $X_i \xrightarrow{c} X_i'$ and $X_i' \xrightarrow{c'} X_i$, where c and c' are interconversion reaction rates. Then the synchronization in other variables X_j and X_j' occurs when the rates c and c' are sufficiently large. In the deterministic limit this corresponds to bidirectional diffusive coupling.
3. **Global or Mean–field coupling**: When a given molecular species, say X_1 is common to a group of systems then this species can be an effective means of global coupling, coupling each system to every other. The common species provides a "mean–field" whereby the systems communicate, and the remaining variables synchronize [40, 39].

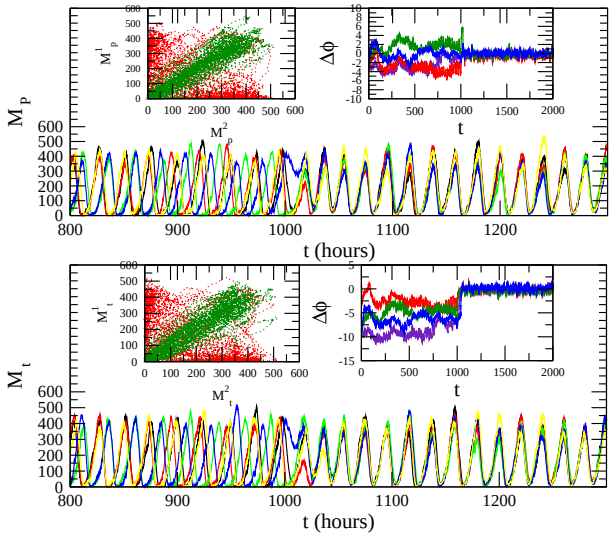

Figure 2: Plot of M_p and M_t populations as a function of time. Direct coupling via P_2 is switched on at $t = 1000$. The left insets show M_p and M_t in cells 1 and 5 before and after coupling, while the right inset show the synchronized and desynchronized regimes.

4. **Noise coupling**: When common noise is added to oscillators, the dynamics of particular species with effective noise, $X_i + \gamma\xi_i$ of one oscillator become synchronized with that of corresponding species, $X_i' + \gamma\xi_i$ induced by strength of the noise, γ, where, ξ satisfies, $\langle \xi_i \xi_j \rangle = \delta_{ij}$ [38]. Interestingly, the two oscillators become correlated at a certain critical value, γ_c, but if $\gamma \langle \gamma_c$, then X_i and X_i' are uncorrelated, whereas if $\gamma \rangle \gamma_c$, the added noise start exploiting the oscillating behaviors and ceases at a particular value.

It has been shown recently [41, 42] that time–delay can induce "relay" synchronization. Systems that are not mutually coupled but instead are both coupled to a third can show either in–phase or out–of–phase synchrony. This form of coupling results in novel temporal patterns, and can give rise to long–range information transfer in a group of oscillators [35]. Such effects are particularly important in the cellular and subcellular domain, since when systems are spatially extended, the coupling can involve time–delay. At a phenomenological level, signals are transmitted via diffusive processes which naturally have a finite velocity. The evolution of such systems can be studied either through delay–differential equations, or through appropriately adapted stochastic simulation techniques [23, 40, 35].

4 Results

To investigate the effecting synchrony in a group of cells, we can either couple them sequentially or simultaneously, namely in series or in parallel. We first present results for the former strategy in the *D. melanogaster* network, using pairwise direct coupling via

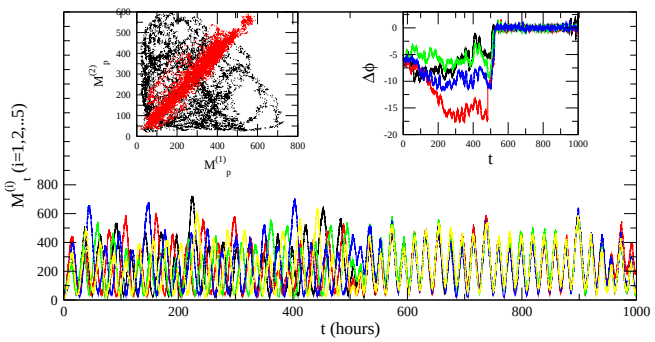

Figure 3: As in Fig. 2, but for the case of *N. crassa*. Coupling is switched on at $t = 500$.

per. Shown in FIG. 2 are the results from our simulations of N=5 cells. The coupling is switched on at time $t = 1000$hr and it can be seen that subsequent to this time, the coupling is very effective in bringing about synchrony. In the regime $t \leq 1000$ hrs the time series of the *per* mRNA, namely M_p populations in the different cells evolve in an uncorrelated fashion. The left inset shows the dependence of the populations on each other, and as synchrony emerges, the dependence becomes linear. The phase difference $\Delta\phi$ of pairs of signals is shown in the right inset, evolving randomly with time in the desynchronized regime, and fluctuating about a constant value once synchrony kicks in. Analogous results for *N. crassa* using *frq* as the driving molecular species can also be obtained as shown in FIG. 3. Here, in this case, coupling is done via *freq*, is switch on at $t = 500$hr.

Synchrony mediated through exchange coupling is also effective in the two cases, and our results are presented in FIG 4 for neuro for different values of the exchange reaction rate, C. For small C the dynamics is uncorrelated, and at higher C synchrony emerges. Shown in the lower right panel shows the variation of the threshold coupling for synchrony as a function of the cellular volume. The transition shares several characteristics of a phase transition, and the behaviour for the *D. melanogaster* system are also similar.

When cells are coupled in the mean field, the correlation between them is stronger and more rapid. Comparison is made with direct coupling, and the results are shown in FIG.5 for *N. crassa*and in FIG.6 for *D. melanogaster*: Synchronization occurs faster and with smaller fluctuations. This may be due to the fact that as the number of communicating molecular species increases, information is transmitted more rapidly.

With delay coupling, both in– and out–of–phase behaviour results. Shown in Fig. 7 are results for coupling five oscillators in series; the upper two panel show the variation of nuclear *per* in cells 1 and 5 as a function of time at coupling constant C=0.1. When the delay time t_d=80, the dynamics is uncorrelated, but for shorter delay t_d=25 the cells synchronize. Similar results are obtained for the case of *N. crassa* (see FIG. 8).

The results of effective noise mediated coupling for two cells in *D. melanogaster*is shown in FIG. 9. In the upper and middle panel shows the results of P_N population for γ=1.4×10^{-3} introducing noise at $t = 1000$hr. Before noise is introduced, the dynamics are uncorrelated, however, as soon as noise is introduced, they become synchronous. But if the strength of the noise is large then the oscillatory behavior of the dynamics become

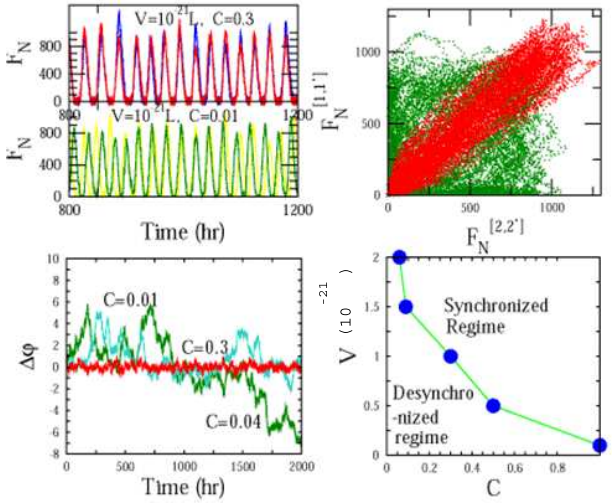

Figure 4: Plot of F_N in two *N. crassa* cells coupled by the exchange of M_F. The upper left panel show the dynamics of F_N for two coupling constant $c = 0.01$ and 0.3 respectively at $V = 10^{-21}L$. The right upper panel and left lower panel show the phase plot. Right lower panel shows phase diagram showing synchronized and desynchronized regimes.

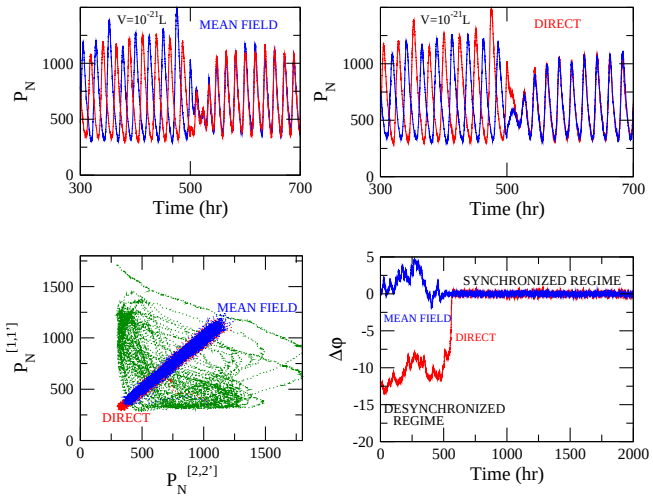

Figure 5: As in Fig. 1, but for the case of mean–field coupling.

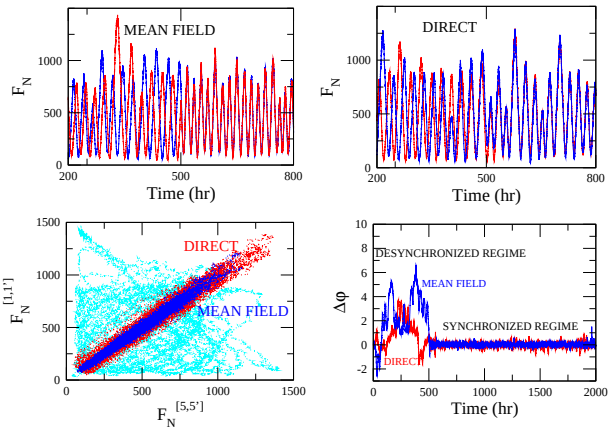

Figure 6: As in Fig. 5, but for the case of *N. crassa*coupling cells 1 and 5.

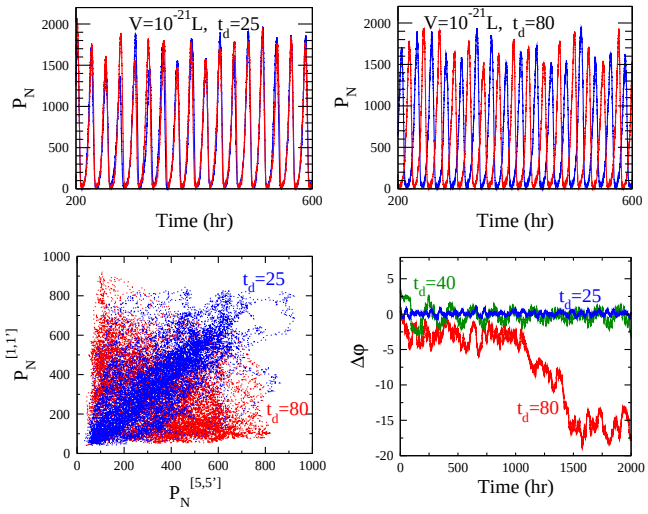

Figure 7: Dynamics of P_N when there is delay in the coupling; here M_P is taken as the coupling molecular species. The upper two panels and lower left panel show P_N in cells 1 and 5, with delays t_d=80 and 25 respectively at C=0.2 and $V = 10^{-21}$L.

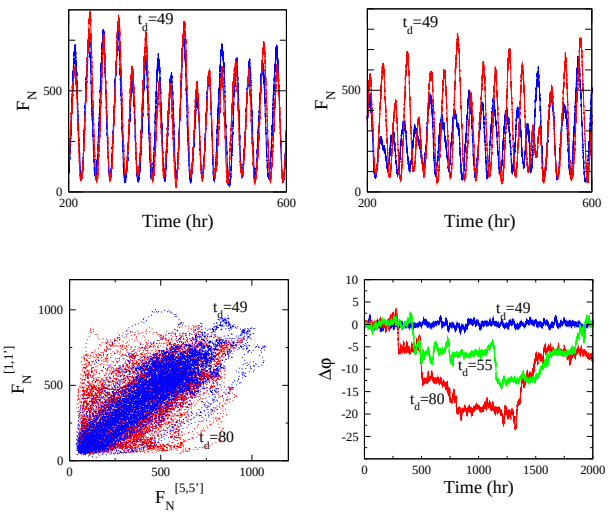

Figure 8: As in Fig. 7, but for the case of *N. crassa*; the corresponding parameters are t_d=80 and 49 at C=0.1 and $V = 10^{-21}$L.

exploited as is shown in middle panel and is for γ=8×10^{-3}. The lowermost panel shows the phase plots of various γs showing synchronised and desynchronized behaviors. Similar results are found for the case of *N. crassa*as is shown in FIG. 10.

We thus observe that the coupling strategies discussed in Section 3 are successful in synchronizing groups of cells in an efficient manner. Global or mean–field coupling is, in a sense, the most robust method that ensures that all the individual oscillators essentially guide each other into a coherent state. Note also that by suitably altering the coupling and delay parameters, arbitrary temporal patterns can be obtained so that it is possible to ensure that groups of cells have complex oscillations. Furthermore, weak noise can enhance correlation of an ensemble of stochastic oscillators. However, if the strength of the noise is large enough, then it may become hinderence to the dynamics of the varibles.

5 Concluding Remarks

We investigated the emergence of synchrony in a group of cells through different coupling mechanisms. Using circadian oscillator models that have been developed for *D. melanogaster* and *N. crassa* and which share a basic common mechanism, we have examined different forms of coupling that are plausible at the molecular level, and furthermore, which cause a rapid synchronization in the dynamics. Again explicit incorporation of time–delay results in relay synchrony between cells with both in– and out–of–phase(or any other relative phase) dynamics being possible. An important aspect of the present work is that we do not consider dynamical models *per se*, but instead treat the individual stochastic processes and couple the different oscillators through elementary reactions. Thus the effect of both internal and external noise is incorporated in the models.

The case of mean–field coupling is of particular significance since a large number of

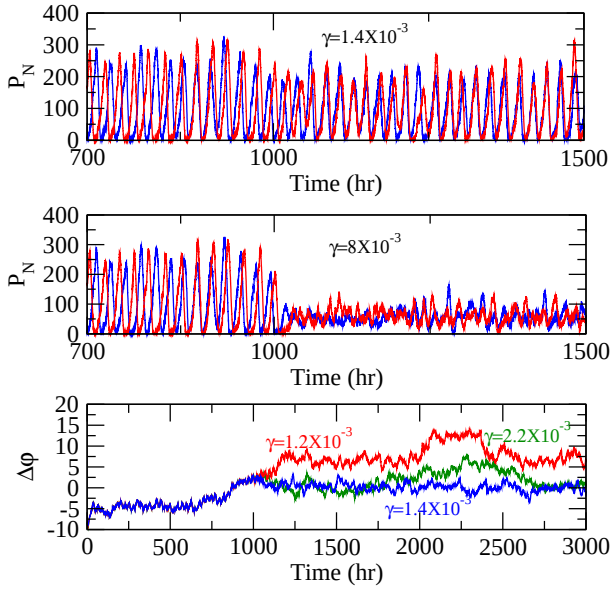

Figure 9: Plot of two *D. melanogaster*cells coupled by effective noise. Upper and middle panel show P_N population as a function of time at $\gamma = 1.4 \times 10^{-3}$ and 8×10^{-3} respectively at $V = 10^{-21}$L. Coupling is switch on at t=1000hr. The lowermost panel shows the phase plots for various noise strengths showing synchronized and desynchronised behaviors.

cells can easily be brought into synchrony through the use of small diffusible molecules, for instance via metabolites. As the number of such species increases, the rate of synchronization also increases. The study of such processes—which are likely to be operative, for instance, in situations where a population of unicellular organisms form a colony—may cast more light on the dynamics of phenomena such as quorum sensing [42]. The addition of noise in such ensemble of cells can lead to synchrony of the dynamics of the variables which gives us important notion that noise can enhance the information processing in the cells even at molecular level. However, if the strength of effective noise is large, the correlated as well as the oscillating behaviors of the dynamical variables are destroyed. Moreover, given the importance of synchronous dynamics in a number of biological systems, study of the manner in which different systems can show a concerted temporal response is of fundamental importance and relevance.

Acknowledgement

RKBS has been supported by the Department of Science and Technology (DST), Government of India under the Fast Track Scheme.

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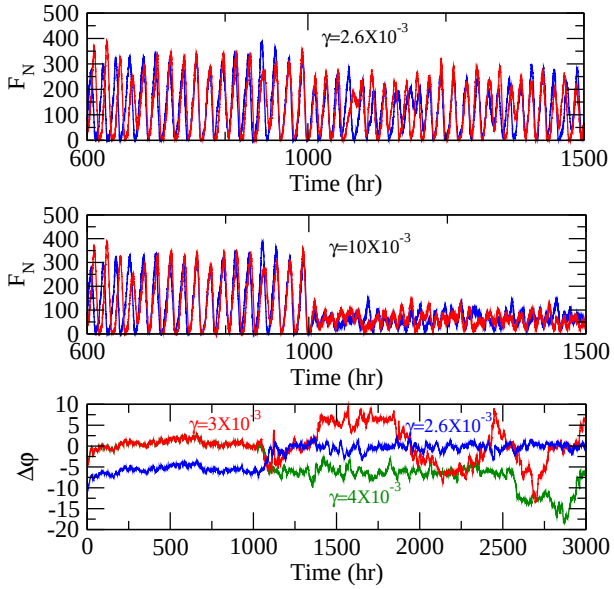

Figure 10: As in Fig. 9, but for the case of *N. crassa*; the corresponding parameters are γ=2.6$\times 10^{-3}$ and 10×10^{-3} at $V = 10^{-21}$L.

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